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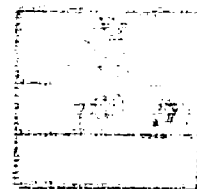
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KINETIC STUDY ON THE MUTAROTATION OF MONOSACCHARIDES BY THE POLAROGRAPHIC METHOD

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List of Frequently Used Symbols

(Unless otherwise denoted)

D_i	:	diffusion coefficient of species i
F	:	Faraday constant
h	:	height of mercury reservoir of dropping mercury electrode
i	:	instantaneous current
\bar{i}	:	average current
\bar{i}_1	:	polarographic average limiting current
\bar{i}_d	:	polarographic average limiting diffusion current
m	:	rate of flow of mercury out of capillary tip
n	:	number of electrons required for electro-reduction of one molecule of depolarizer
q	:	surface area of electrode
\bar{q}	:	mean surface area of electrode
R	:	gas constant
T	:	absolute temperature
μ	:	thickness of reaction layer
$\bar{\mu}$:	mean thickness of reaction layer
τ	:	drop time

INTRODUCTION

In 1933, Heyrovsky, Smoler, and Stastny¹⁾ reported that D-glucose was reduced at the dropping mercury electrode. This observation was the first description of the polarography of sugar. The temperature coefficients of the limiting current were about 10- to 15-times larger than those for diffusion-controlled currents. Relatively high concentrations (ca. 0.1M) were required and the limiting currents observed were low. Later in 1940 Cantor and Peniston²⁾ carried out an extensive study on polarography of several sugars. It was believed that the aldehyde-form of the sugar was in equilibrium with a much larger amount of the ring form and that the aldehyde-form alone was reducible so that only low limiting current, controlled by the rate of diffusion of this form to the drop surface, could be expected. On the basis of this assumption the amount of the aldehyde-form present in solutions of several aldohexoses and aldopentoses was determined by the polarographic method.

However, Sugino and Hayashi³⁾ found that the wave-height of D-glucose, D-mannose, and D-xylose increased with increasing pH of the solution. This observation led them to suppose that the wave-height is limited by the conversion velocity of the nonreducible ring form to the reducible aldehyde form. In 1947, Wiesner⁴⁾ paid his attention to the characteristics of the limiting current for several aldoses and demonstrated that the limiting current of such waves were independent of the height of the mercury reservoir. Wiesner accordingly concluded that the limiting current was determined, not by the rate of diffusion of the aldehyde-form to the mercury drop, but by the rate of transformation of the α - β -equilibrium mixture from the ring to the open-chain reducible form at the surface of the mercury drop. He showed that the equilibrium concentration of the aldehyde-form was negligible. The values of the rate of this transformation were estimated for several monosaccharides.^{4,5,6,7)}

In later papers, Wiesner and coworkers⁸⁾ found that the kinetic

current of freshly dissolved α -D-glucose decreased with time to a constant value, which was reached after several minutes. They assumed the following mutarotation equilibrium to explain this phenomenon: α -glucose $\xrightleftharpoons[k_{-1}]{k_1} \gamma \xrightleftharpoons[k_2]{k_{-2}}$ β -glucose, where γ denotes the reducible free aldehyde-form of glucose; and they determined the rate constants, k_1 , k_2 , k_{-1} , and k_{-2} .

On the other hand, the attempt to express the kinetics of mutarotation reaction of sugars was made by many workers in the field of carbohydrate chemistry. Hudson^{9,10)} formulated the mutarotation reaction of sugars as a reversible reaction: α -form $\xrightleftharpoons[k_\beta]{k_\alpha}$ β -form and the velocity as $dx/dt = k_\alpha(a-x) - k_\beta(x)$, which, upon integration, becomes: $k_\alpha + k_\beta = 1/t \ln[Ka/(Ka - (1+K)x)]$, where $(k_\alpha + k_\beta)$ is the mutarotation constant, and $K = k_\alpha/k_\beta$. The mutarotation constant, $(k_\alpha + k_\beta)$, is the sum of the constants for the two opposing reactions, and k_α/k_β is the equilibrium constant. Lowry¹¹⁾ and Hudson^{9,10)} pointed out and showed that the same value should be and was obtained for $k_\alpha + k_\beta$ from the mutarotation measurements of the α - and β -anomers. Hudson found the reaction constant to be independent of the concentration of sugar over a wide range, and dependent on catalysis by both acids and bases.

Brönsted and Guggenheim^{12,13)} concluded that the mutarotation requires an acid catalyst and/or a base catalyst and that amphoteric solvents are complete catalysts for the process, whereas aprotic solvents are not. They also showed that molecules of undissociated acids, cations of weak bases, and ions of weak acids have catalytic properties. This concept became to be known as generalized acid and base catalysis. It was found that the rate of mutarotation of a sugar in the presence of a mixture of several catalysts may be represented by an equation of the type: $(k_\alpha + k_\beta) = k_{H_2O}[H_2O] + k_{HA,j}[HA_j] + k_{B,n}[B_n]$, where the symbols in brackets represent the concentrations (activities) of the catalysts, and the coefficients, $k_{HA,j}$ and $k_{B,n}$, represent the catalytic activity of the acid and base catalysts, respectively.

Numerous workers have examined the rate constants for the mutarotation of sugars by the polarimetric method.^{14,15,16)} A number of other methods have also been used for following the mutarotation reaction kinetically. These include changes of: volume, refractive index, infrared absorption,¹⁷⁾ calorimetric properties,¹⁸⁾ pH,^{19,20)} and gas-liquid chromatographic behavior.²¹⁾ Recently, nuclear magnetic resonance measurements has also been used.²²⁾

On the basis of these observations, several mechanisms for mutarotation of sugars in aqueous solution have been proposed.¹⁵⁾ It is relatively well established that the mutarotation of a sugar in aqueous solution involves transfer of a proton from an acid catalyst to the sugar, and the transfer of another proton from the sugar to a base catalyst. The reaction starts with attack on the cyclic sugar by either an acid or a base catalyst, followed by a slow rupture of the ring. In the resulting intermediate, reaction of the hydroxyl group on C-5 of aldoses yields the anomeric aldopyranoses. The sequence and the timing of the addition and elimination of the protons give rise to several reaction-paths.

The specific mechanism of this reaction, however, has not been made sufficiently clear. Differences in the ionization constant^{19,20)} for α - and β -anomer, and possible differences in the transition states for the anomers, make detailed investigations of the individual velocity constants, k_α and k_β , desirable. The methods mentioned above, however, can be used largely to determine the overall velocity constant, $k_\alpha + k_\beta$ for the reversible reaction: α -pyranose $\xrightleftharpoons[k_\beta]{k_\alpha}$ β -pyranose.

On the contrary, by the use of polarography we can detect the reducible intermediate, γ . Thus, it becomes possible to determine the individual velocity constants as shown first by Wiesner and his coworkers.⁸⁾ In view of this advantage of the polarographic method, the author's attention has been concentrated to the polarographic behavior of sugar, especially in connection with the kinetics of mutarotation.

In this thesis, a contribution of the author to the polarography of monosaccharides has been summarized in five chapters. The first chapter is concerned with detailed studies on the polarographic behavior of α - β -equilibrated monosaccharides in basic buffer solutions. In the second chapter, the equations, relating the polarographic kinetic current with the rate constants of mutarotation are derived and the rate constants for several monosaccharides are determined by use of these equations. The third chapter deals with the acid-base catalyzed mutarotation of D-xylose. In the fourth chapter, the reaction mechanism of acid-base catalyzed mutarotation of monosaccharides is discussed. The last chapter describes the polarographic behavior of monosaccharides in an unbuffered aqueous solution and its application to the determination of the mutarotation velocity.

CHAPTER I. POLAROGRAPHIC BEHAVIOR OF α - β -EQUILIBRATED MONOSACCHARIDES IN BASIC BUFFER SOLUTIONS

Many polarographic studies of α - β -equilibrated monosaccharides have been carried out in neutral or basic solutions,^{1-7,23.24)} but not in acidic solutions. In acidic solutions, a polarographic wave of monosaccharide cannot be observed because of the final ascending of the residual current due to the reduction of hydrogen ion on the dropping mercury electrode. A monosaccharide is stable in acidic or neutral aqueous solutions at a room temperature; but in basic solutions, it is unstable even under the mild conditions. Lobry de Bruyn rearrangement, the fragmentation, and the other complicated reactions may occur.²⁵⁾ In addition, the carbonyl-amino reaction may also occur in amine-buffered basic solutions.

In this chapter is described the electrochemical behavior of D-glucose, D-galactose, and D-xylose, as studied by polarography and controlled-potential electrolysis, especially in weakly basic solutions.

EXPERIMENTAL

Materials The D-glucose, D-galactose, and D-xylose of reagent grade were purchased from Nakarai Chemical Company and used without further purification. Ammonium chloride and potassium hydroxide were used for preparing ammonia buffer solutions. Potassium chloride, was used to adjust the ionic strength to any desired values (usually 0.5). All chemicals were reagent grade and used without further purification.

Apparatus A Yanagimoto polaro-recorder, type PA-103, was used for all polarographic measurements. A Yanagimoto controlled-potential

electrolyzer, type VE-3, was used for controlled-potential electrolysis, Polarimetric measurements were carried out with a Yanagimoto polarimeter, type OR-20. PH values were measured with a Hitachi Horiba M-5 pH meter.

The polarographic measurements were made with an H-type cell; it was connected with a saturated calomel electrode (SCE) by means of an agar-gelatine bridge containing potassium chloride. The capillary characteristics of the dropping mercury electrode (open circuit) at a mercury reservoir height of 62.5 cm were $m = 1.550 \text{ mg sec}^{-1}$ and $\tau = 5.74 \text{ sec}$. Stock solutions of monosaccharides were prepared with bidistilled water. Each experimental solution was made up as follows; an aliquot of sugar solution was mixed with an ammonia buffer solution and diluted with water to make an electrolyte solution of a desired concentration of the sugar and the buffer component. Potassium chloride was added, if necessary, to adjust the ionic strength to a desired value. An experimental solution was freed of oxygen by passing a nitrogen stream through the solution. The polarogram was recorded after the α - β -equilibrium of the sugar had been reached (10-15 mins.). The nitrogen stream was passed over the solution during the measurement. Experiments were usually carried out in a water thermostat controlled at $25 \pm 0.05^\circ\text{C}$.

RESULTS AND DISCUSSION

Stability of monosaccharide in weakly basic buffer solutions

The time dependence of the specific rotation, pH, and the polarogram of the sugar solution containing 0.5 M ammonia buffer (pH 9.50) was examined at 25°C . As shown in Table I-1, no appreciable change in specific rotation as well as pH of α - β -equilibrated solutions of monosaccharides was observed, at least within three hours at 25°C . No appreciable change in the polarogram was observed, either. These results suggest that hydrolysis, carbonyl amino reaction, or any

other complicated reactions of monosaccharides will not occur to any appreciable extent under the conditions used in the present experiment. As an example, the time dependence of the polarogram of D-glucose is shown in Fig. I-1.

TABLE I-1. TIME DEPENDENCE OF $[\alpha]_D^{25}$, pH, AND \bar{i}_1 , OF THE SUGAR SOLUTION CONTAINING 0.5M $\text{NH}_3\text{-NH}_4^+$ AT 25°C

Time	D-glu.			D-gal.			D-xyl.		
	$[\alpha]_D^{25}$	pH	\bar{i}_1	$[\alpha]_D^{25}$	pH	\bar{i}_1	$[\alpha]_D^{25}$	pH	\bar{i}_1
			(cm)			(cm)			(cm)
10 min.	52.4	9.50	5.51	79.8	9.50	5.01	18.9	9.50	5.65
1 hr.	52.4	9.50	5.50	80.4	9.50	5.05	18.9	9.50	5.65
2 hrs.	52.2	9.50	5.50	79.0	9.50	4.95	18.0	9.50	5.60
3 hrs.	52.2	9.50	5.49	79.8	9.50	5.05	18.9	9.50	5.65

$[\alpha]_D^{25}$: Specific rotation, \bar{i}_1 : Limiting current (current sensitivity 1μA/cm) D-glu.: 0.30M, D-gal.: 0.08M, D-xyl.: 0.06M.

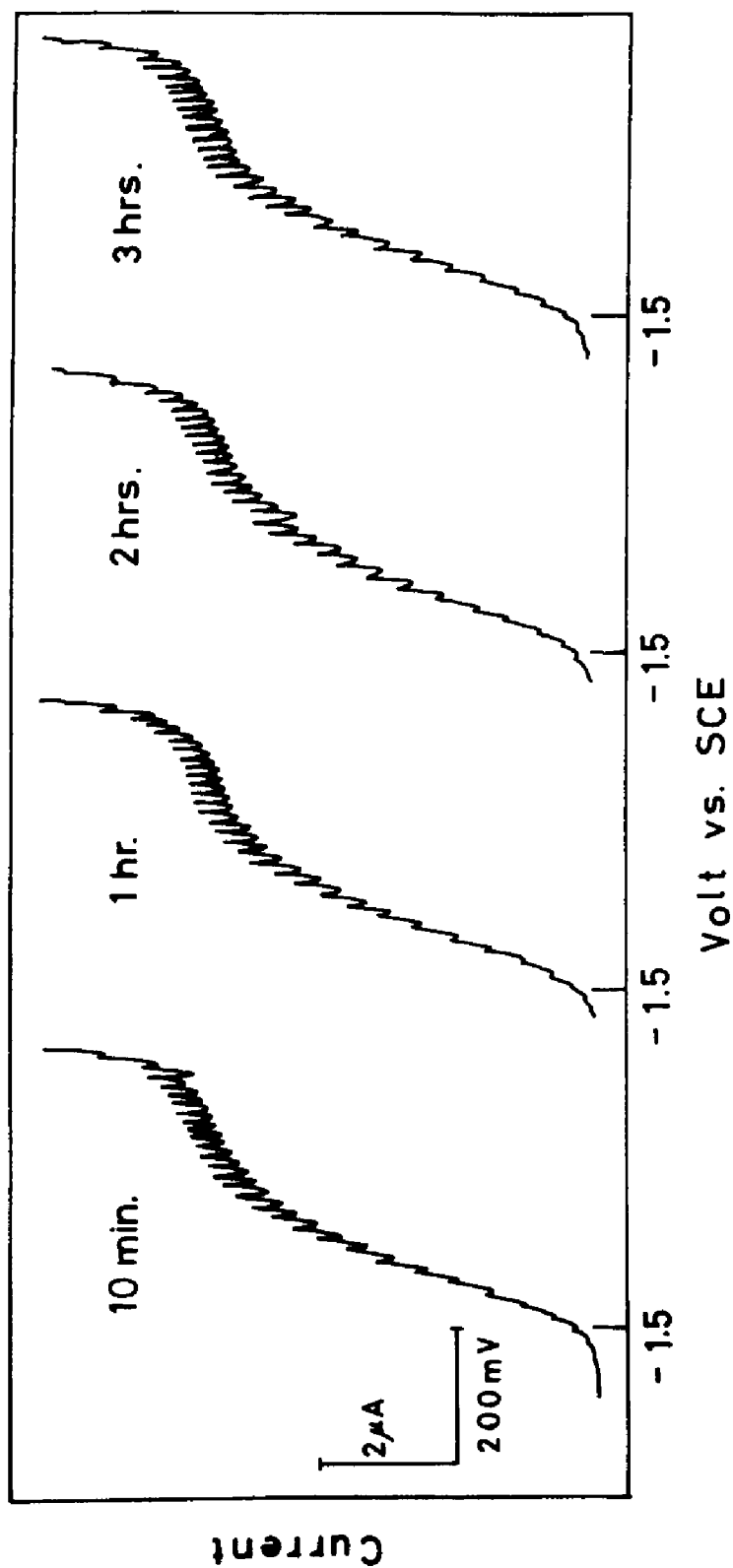
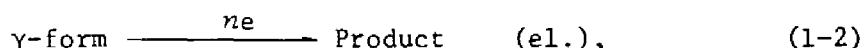
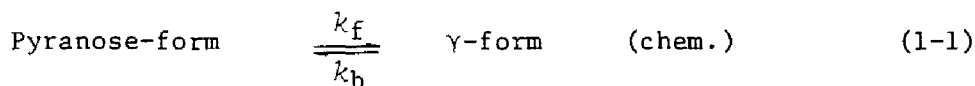


Fig.I-1 Time dependence of the polarogram of D-glucose (0.30 M)
in 0.5 M ammonia buffer solution of pH 9.50 at temp. 25°C.

The limiting current of the polarographic wave of monosaccharides

The reaction schemes (1-1) and (1-2) can be assumed for the reduction of α - β -equilibrium mixture of monosaccharide:⁵⁾



where γ represents the reducible intermediate. The limiting kinetic current, \bar{i}_1 , is then shown to be given by Eq.(1-3):

$$\bar{i}_1 = \bar{i}_d \frac{\tau^{1/2}}{1.23} \frac{k_f}{k_b^{1/2}}, \quad (1-3)$$

under the conditions:²⁶⁾

$$\tau(k_f + k_b) \gg 1, \text{ and } k_b/k_f \gg 1, \quad (1-4)$$

where τ is the drop time, \bar{i}_d the hypothetical diffusion current given by $\bar{i}_d = 607nm^{2/3} \tau^{1/6} D^{1/2} C_{\text{sugar}}$, D the diffusion coefficient of the sugar and C_{sugar} the total concentration of the sugar. Eq. (1-3) predicts that \bar{i}_1 is proportional to the concentration of sugar and independent of the height of mercury reservoir, h .

Effect of Sugar Concentration on Limiting Current: As shown in Fig.I-2, the value of \bar{i}_1 is directly proportional to the total concentration of the sugar. The slope of this line reflects the velocity of the reaction given by the scheme (1-1).

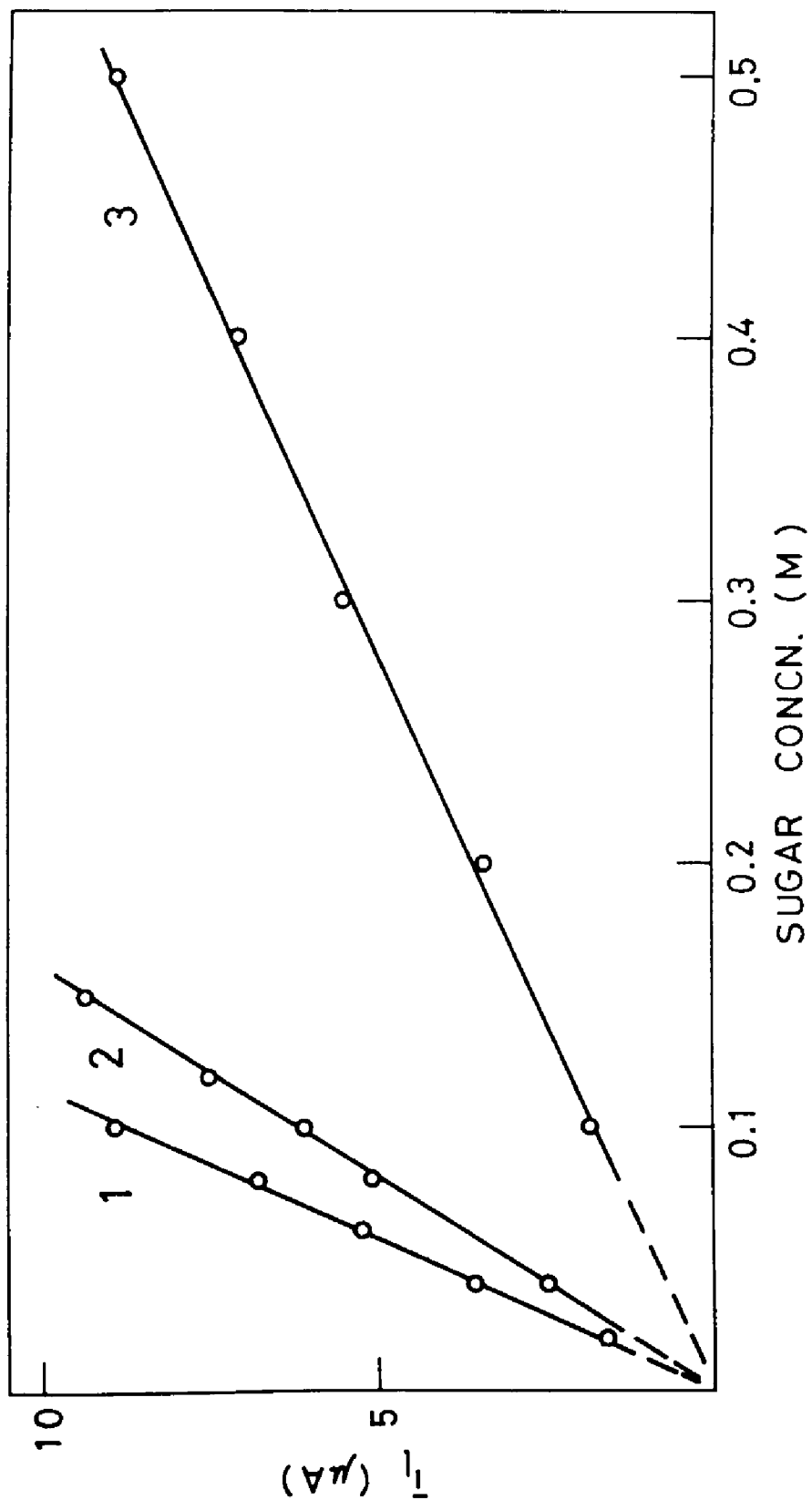


Fig.I-2 Dependence of the limiting current on the concentration of monosaccharides in 0.5M ammonia buffer solution of pH 9.50 at temp. 25°C; 1: D-xylose, 2: D-galactose, 3: D-glucose.

Actually the values of this slope decrease in the order of D-xylose > D-galactose > D-glucose and this order corresponds to that of the mutarotation rates in aqueous solution.¹⁴⁾

Effect of Temperature: Fig. I-3 shows the dependence of \bar{i}_l on the temperature. All of these sugars show about the same temperature coefficient (ca. 10 %), which is about ten times larger than that of the diffusion controlled limiting current.

Effect of Mercury Reservoir Height: The limiting current was inclined to increase slightly with an increase in the height of the mercury reservoir (Table I-2).

TABLE I-2. DEPENDENCE OF THE LIMITING CURRENT, \bar{i}_l , ON THE HEIGHT OF MERCURY RESERVOIR, h AT 25°C

h	\bar{i}_l (cm)		
(cm)	D-glu.	D-gal.	D-xy1.
42.5	5.50	5.00	5.60
52.5	5.54	5.01	5.63
62.5	5.57	5.10	5.65
72.5	5.60	5.25	5.80
c.f. \bar{i}_l/\bar{i}_d	4.0×10^{-3}	1.4×10^{-2}	2.1×10^{-2}

D-glu.: 0.30M, D-gal.: 0.08M, and D-xy1.: 0.06M
in the ammonia buffer (0.5M) of pH 9.50. Current
sensitivity: 1μA/cm.

This result is not rigorously in accord with the prediction from Eq.(1-3). Table I-2 also shows that the ratio of the limiting current to the hypothetical diffusion current, \bar{i}_1/\bar{i}_d , is very small ($10^{-2} - 10^{-3}$). These results suggest that the equilibrium concentration of the intermediate, γ , is very small in comparison with the concentration of the pyranose form, but the reaction given by Eq. (1-1) is slow, so that the condition, $\tau(k_f + k_b) \gg 1$, is not necessarily satisfied. This point will be discussed in Chapter II.

Effect of PH and Buffer Concentration: Fig. I-4 shows the pH dependence of \bar{i}_1 at a given buffer concentration. The value of \bar{i}_1 increases exponentially with the pH. Table I-3 shows that the

TABLE I-3. LIMITING CURRENT, \bar{i}_1 , WITH VARIOUS BUFFER CONCENTRATIONS AT CONSTANT pH (8.84) AT 25°C

Concn. of ammonia buffer (M)	\bar{i}_1 (cm)		
	D-glu. (0.30 M)	D-gal. (0.10 M)	D-xyl. (0.04 M)
0.030	4.47	3.68	2.72
0.059	4.89	4.17	2.96
0.117	5.17	4.87	3.24
0.233	6.01	5.74	3.66
0.466	7.07	7.07	4.26

Ionic strength 0.5. Current sensitivity 0.6 μ A/cm.

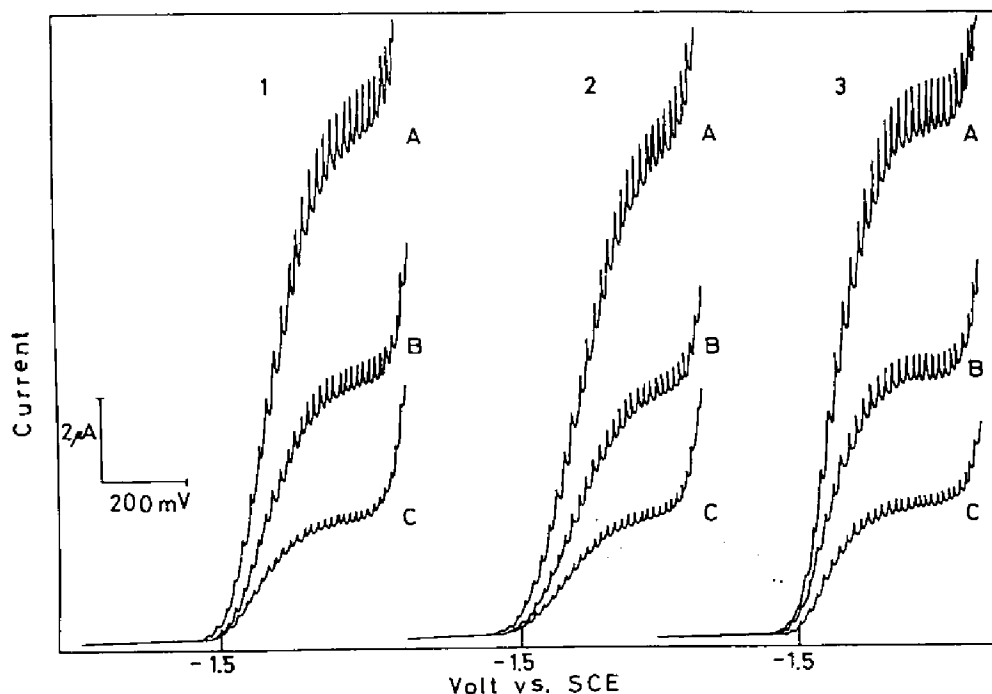


Fig.I-3. Effect of temperature on the polarograms of monosaccharide in 0.5 M ammonia buffer solution $[(\text{NH}_4^+)/(\text{NH}_3)=3/2]$; 1: D-glucose, 2: D-galactose 3: D-xylose. A: 35°C, B: 25°C, C: 15°C.

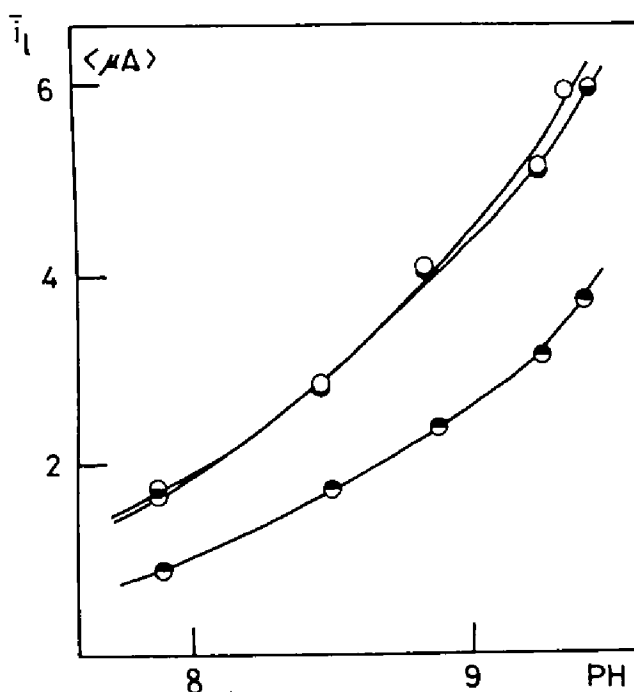


Fig.I-4 Dependence of the limiting current on the pH at a constant buffer concentration (0.5 M ammonia buffer); ○: D-glucose (0.30M) ●: D-galactose (0.10M) ●: D-xylose (0.04M). Temp. 25°C.

limiting current increases with increasing concentration of the buffer component at a given pH. These results suggest that the reaction given by Eq.(I-1) is catalyzed by the hydroxyl ion and by the buffer components as well.

Identification of the reduction product *Controlled-potential Electrolysis of D-Glucose, D-Galactose, and D-Xylose in Basic Solution:* Sugino and Hayashi³⁾ have reported that D-glucose is reduced to sorbitol in basic solutions (pH<10), but at higher pH value than 10 where D-fructose is produced from D-glucose, mannitol in addition to sorbitol is produced by the electro-reduction of D-glucose. In this study controlled-potential electrolysis of monosaccharides was carried out at the mercury pool cathode with 50 mM sugar solution in an ammonia buffer solution (pH 9.5, $\mu=0.5$, adjusted with KCl). The applied potential was -1.8 V vs. SCE for all monosaccharides, at which potential the limiting current was obtained. Five ml of the sugar solution was electrolyzed. The cell arrangement for the electrolysis is shown in Fig. I-5.

After about nine hours of electrolysis at -1.8 V vs. SCE, the kinetic wave of monosaccharides disappeared almost completely (Fig.I-6). During the electrolysis, generation of bubbles was observed on the mercury-cathode surface. These bubbles were also observed in controlled-potential electrolysis of the buffer solution in which no monosaccharide was contained. Accordingly these bubbles may be attributed to the evolution of hydrogen, which may occur parallel with the reduction of monosaccharide. Provided that the number of electrons consumed per molecule of the monosaccharide is 2, calculation showed that about two-thirds of the total electricity was consumed by the reduction of the monosaccharide and one third by the reduction of hydrogen ion.

Paper-chromatography of Electrolyzed Product: Paper-chromatography was carried out on Toyo filter paper No 51 A using a solvent system containing butanol, ethanol, and water (4:1:4). The spots were detected by two kinds of colour-producing reagents;²⁷⁾

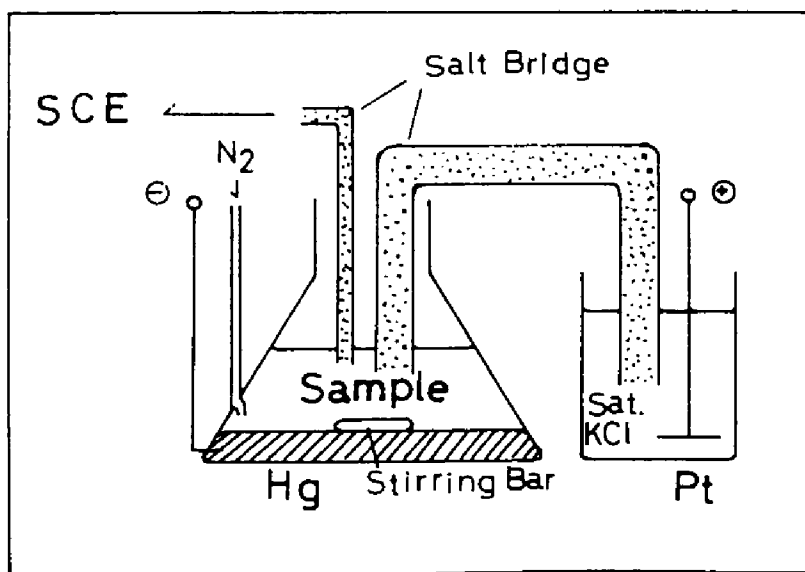


Fig.I-5 Cell arrangement for the controlled-potential electrolysis.

the first reagent(reagent I) was 1 % KMnO_4 containing 2 % Na_2CO_3 , by which sugar alcohol as well as sugar is yellow coloured. The second reagent(reagent II) was aniline hydrogen phthalate, by which sugar is coloured, but sugar alcohol is not.

D-glucose: D-glucose solutions before and after the electrolysis were paper-chromatographed together with the authentic mannitol and sorbitol. All of them gave each one spot of yellow colour by reagent I. The electrolyzed solution gave an spot with the same R_f value as that of the authentic sorbitol. A brownish colour was developed for the D-glucose solution before the electrolysis by reagent II, but did not for the electrolyzed solution and the authentic sorbitol solution. These results indicate that the electrolyzed product is sorbitol.

D-galactose: D-galactose solutions before and after the electrolysis were paper-chromatographed together with the authentic dulcitol. All of them gave each one spot of yellow colour by reagent I. The electrolyzed solution gave a spot with the same R_f value as

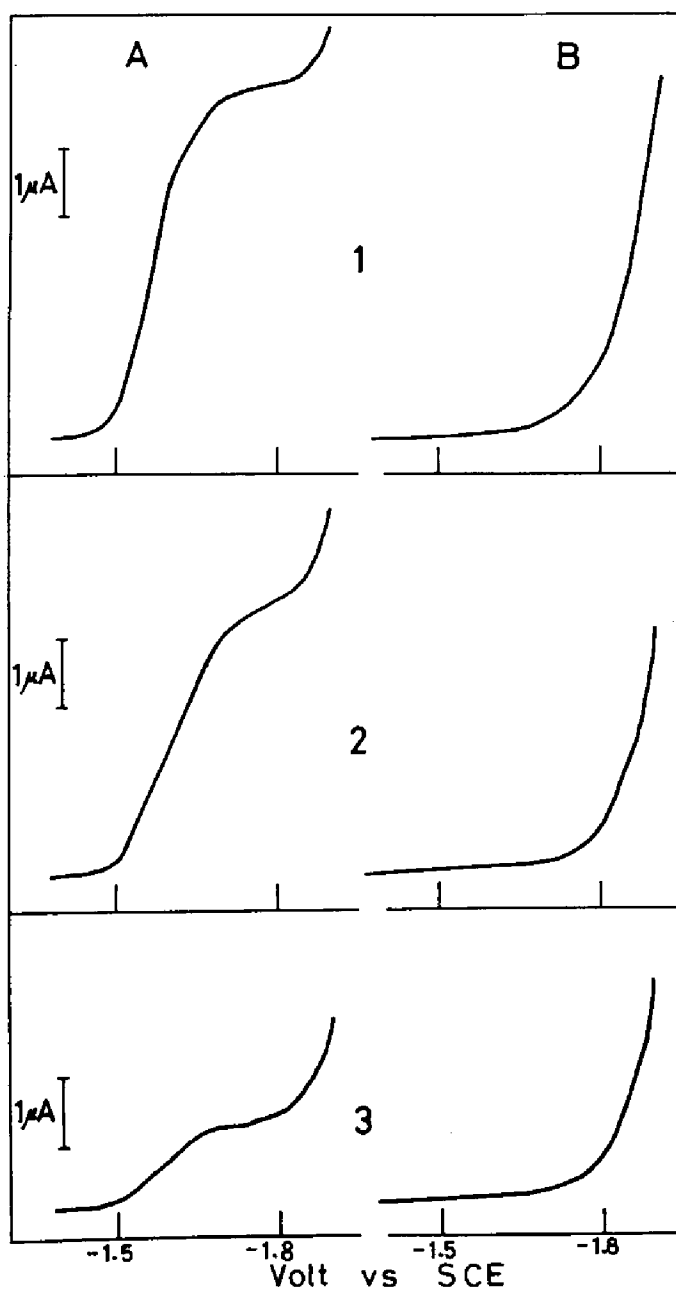


Fig.I-6 Polarograms of monosaccharides (1: D-xylose, 2: D-galactose, and 3: D-glucose) before the electrolysis (A) and after about nine hours of electrolysis (B) at mercury pool cathode (-1.8 Volt vs. SCE). Temp. 25°C.

that of the authentic dulcitol. A brownish colour was developed only for D-galactose solution before the electrolysis by reagent II. These results indicate that the electrolyzed product is dulcitol.

D-xylose: D-xylose solution before and after the electrolysis were paper-chromatographed together with the authentic xylitol. All of them gave each one spot of yellow colour by reagent I. The electrolyzed solution gave a spot with the same R_f value as that of the authentic xylitol. A reddish colour was developed only for D-xylose solution before the electrolysis by reagent II. These results indicate that the electrolyzed product is xylitol.

SUMMARY

The polarographic behavior of α - β -equilibrated mixture of D-glucose, D-galactose, and D-xylose were studied in an ammonia buffer solution. Hydrolysis, carbonyl amino reaction, or any other complicated reactions of these monosaccharides did not occur under the conditions used in the present experiment (pH<9.5, Temp. 25°C).

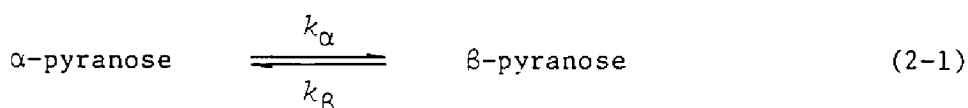
The dependence of the limiting current on the concentration of monosaccharides, temperature, the height of mercury reservoir, the pH, and the concentration of buffer components was investigated. The polarographic limiting current had the kinetic character, but it was inclined to increase slightly with increasing height of the mercury reservoir. The limiting current depended on the concentration of the buffer component as well as the pH of the experimental solution.

From these results, it was concluded that the equilibrium concentration of the reducible intermediate is very small in comparison with the concentration of the pyranose form; and the reaction given by Eq.(I-1) is catalyzed by the buffer component as well as hydroxyl ion.

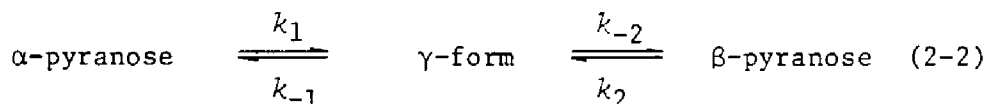
The controlled-potential electrolysis of the monosaccharides and the paper-chromatography of the electrolyzed solutions revealed that D-glucose, D-galactose, and D-xylose are reduced to sorbitol, dulcitol, and xylitol, respectively at the mercury cathode in weakly basic solution.

CHAPTER II. DETERMINATION OF MUTAROTATION RATE CONSTANTS OF MONOSACCHARIDES BY THE POLAROGRAPHIC METHOD

The mutarotation of reducing sugar in an aqueous solution is catalyzed by acids and bases.^{15,16)} The measurements of the mutarotation velocity have been usually made by polarimetry,²⁸⁻³⁷⁾ NMR,²²⁾ and others,^{20,21,38,39)} but the results are in most cases interpreted in terms of the mutarotation rate constant, k_0 , defined by $k_0 = k_\alpha + k_\beta$, where the reaction mechanism is assumed to be as Eq.(2-1):



Los, Simpson, and Wiesner⁸⁾ have shown that, by the use of the polarographic method, four individual rate constants, k_1 , k_2 , k_{-1} , and k_{-2} , of the mutarotation assumed as in Eq.(2-2):



can be determined; where the intermediate γ -form is assumed to be an electroactive free aldehyde form, and the polarographic current produced by D-glucose is controlled by the kinetics of the formation of the γ -form from α - and β -pyranose. Experimental study of the isotope exchange of D-glucose C-1-¹⁸O¹⁴⁾ also supports the idea that the formation of aldehydrol form is negligible in mutarotation kinetics.

Los, Simpson, and Wiesner's method, however, cannot be applied for the case of small values of the velocity constants. In this chapter, the author derives modified equations which can be applied for the system with smaller values of the rate constants. The rate constants of D-glucose, D-galactose, and D-xylose in neutral and weakly basic buffer solutions are determined by use of the modified equations.

II-1. EXPRESSION FOR THE POLAROGRAPHIC CURRENT OF MONOSACCHARIDES

According to Los, Simpson, and Wiesner⁸⁾ the polarographic limiting current, \bar{i}_1 , of monosaccharide when it is present in two forms, *e.g.*, α -pyranose and β -pyranose at the concentrations of C_α and C_β , is given by:

$$\bar{i}_1 = nF\bar{q} \left(\frac{D}{k_{-1} + k_{-2}} \right)^{1/2} (k_1 C_\alpha + k_2 C_\beta) \quad (2-3)$$

Eq.(2-3) was first derived by assuming a reaction layer on the electrode surface, the thickness of which was given by:

$$\mu = \left(\frac{D}{k_{-1} + k_{-2}} \right)^{1/2} \quad (2-4)$$

M.Senda²⁶⁾ has shown that Eq.(2-3) can be derived by a rigorous mathematical procedure on the following assumption:

$$\tau(k_{-1} + k_1) \gg 1, \quad \tau(k_{-2} + k_2) \gg 1, \quad (2-5)$$

and

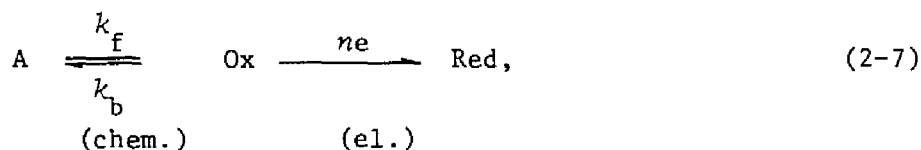
$$K_\alpha = k_{-1}/k_1 \gg 1, \quad K_\beta = k_{-2}/k_2 \gg 1, \quad (2-6)$$

while k_1/k_2 is not extremely different from unity. This is actually the case with D-glucose in a phosphate buffer solution.⁸⁾ A similar argument has also been advanced by Paldus and Koutecky.⁴⁰⁾

As will be shown later in this chapter, however, the rate constants for monosaccharides in basic buffer solutions are not always so large as Eq.(2-5) holds. Accordingly, a modified equation has to be used in analyzing the polarographic kinetic current of monosaccharides in weakly basic buffer solutions.

By close investigation of the rigorous mathematical solution for the polarographic kinetic current reported by Koutecky and Brdicka,⁴¹⁾ and by assuming the reaction layer given by Eq.(2-4), we may derive a modified equation of the polarographic current.

The expression for the instantaneous limiting current, $i(t)$, of an electrode reaction with a preceding chemical reaction;



at a stationary plane electrode has been given by:⁴¹⁾

$$\begin{aligned} \frac{i(t)}{nFq} = \frac{D^{1/2}C^*}{\pi^{1/2}(K^2-1)} & \left[\frac{Ke^{-lt} - 1}{t^{1/2}} + \frac{K^2(\pi k_f)^{1/2} e^{\frac{k_f t}{K-1}}}{(K-1)^{1/2}} \right. \\ & \left. \times \left(\text{erf}\left(K \left(\frac{tk_f}{K-1}\right)^{1/2}\right) - \text{erf}\left(\frac{tk_f}{K-1}\right)^{1/2} \right) \right] \end{aligned} \quad (2-8)$$

where C^* is the sum of the concentrations of A and Ox; $K = k_b/k_f$ and $l = k_f + k_b$.

When $K \gg 1$, and lt is in the order of unity or less, so that $(k_f t/K)^{1/2} \approx (lt)^{1/2}/K$, $K \exp(-lt) \gg 1$, $\text{erfc}((lt)^{1/2}/K) \approx 1$, and $\exp(lt/K^2) \approx 1$, Eq.(2-8) may be reduced in a good approximation to:

$$\begin{aligned} \frac{i(t)}{nFq} = \frac{D^{1/2}C^* l^{1/2}}{K} & + \frac{D^{1/2}C^*}{K(\pi t)^{1/2}} \exp(-lt) \times \\ & \times (1 - (\pi lt)^{1/2} \exp(lt) \text{erfc}(lt)^{1/2}) \end{aligned} \quad (2-9)$$

Accordingly, the mean limiting current, \bar{i}_1 , at the dropping mercury electrode is given by:

$$\bar{i}_1 = \frac{1}{\tau} \int_0^{\tau} nFq \frac{D^{1/2} C^* l^{1/2}}{K} dt + \frac{1}{\tau} \int_0^{\tau} nFq \frac{D^{1/2} C^*}{K(\pi t)^{1/2}} \times \exp(-lt) [1 - (\pi lt)^{1/2} \exp(lt) \operatorname{erfc}(lt)^{1/2}] dt \quad (2-10)$$

The second term on the right hand side of Eq.(2-10) will be given in the first approximation for a smaller value of $l\tau$ by:

$$g_2(\tau) = \frac{1 - \exp(-l\tau)}{l\tau} \left[\frac{1}{\tau} \int_0^{\tau} nFq \frac{D^{1/2} C^*}{K(\pi t)^{1/2}} dt - \frac{1}{\tau} \int_0^{\tau} nFq \frac{D^{1/2} C^*}{K} l^{1/2} \exp(lt) \operatorname{erfc}(lt)^{1/2} dt \right] \quad (2-11)$$

Furthermore, according to Matsuda⁴²⁾ or Koutecky,⁴³⁾ the second term in the brackets on the right-hand side of Eq.(2-11) should be replaced by $(\bar{i}_d/K)(1+1.13(l\tau)^{-1/2})$ for the case of the dropping mercury electrode. Thus, the mean current, \bar{i}_1 , at the dropping mercury electrode may be given by:

$$\bar{i}_1 = 0.81 \frac{(l\tau)^{1/2}}{K} \bar{i}_d + \frac{\bar{i}_d}{K} \frac{1 - \exp(-l\tau)}{l\tau} \left(1 - \frac{1}{1.13(l\tau)^{-1/2} + 1} \right) \quad (2-12)$$

where \bar{i}_d is the diffusion current given by Ilkovic equation with the total bulk concentration, C^* .

If we proceed with the reaction scheme (2-2), $K_\alpha + K_\beta$ and $k_{-1} + k_{-2}$ should be substituted for K and l , respectively.²⁶⁾

In conclusion, we obtain Eq.(2-13):

$$\bar{z}_1 = 0.81 \frac{[\tau(k_{-1} + k_{-2})]^{1/2}}{K_\alpha + K_\beta} \bar{z}_d + \frac{\bar{z}_d}{K_\alpha + K_\beta} \frac{1 - \exp[-\tau(k_{-1} + k_{-2})]}{\tau(k_{-1} + k_{-2})}$$

$$\times \left(1 - \frac{1}{1.13(\tau(k_{-1} + k_{-2}))^{-1/2} + 1} \right) = \bar{z}_k + \bar{z}_{corr} \quad (2-13)$$

with

$$\bar{z}_k = 0.81 \frac{[\tau(k_{-1} + k_{-2})]^{1/2}}{K_\alpha + K_\beta} \bar{z}_d \quad (2-14)$$

$$\bar{z}_{corr} = \frac{\bar{z}_d}{K_\alpha + K_\beta} \frac{1 - \exp[-\tau(k_{-1} + k_{-2})]}{\tau(k_{-1} + k_{-2})} \left(1 - \frac{1}{1.13(\tau(k_{-1} + k_{-2}))^{-1/2} + 1} \right) \quad (2-15)$$

$$\bar{z}_d = \kappa(C_\alpha + C_\beta) \quad \kappa : \text{Ilkovic constant} \quad (2-16)$$

under the following conditions:

$$[\tau(k_{-1} + k_{-2})]^{1/2} / (K_\alpha + K_\beta) \ll 1 \quad (2-17)$$

and

$$K_\alpha \gg 1, \quad K_\beta \gg 1 \quad (2-18)$$

The first term on the right-hand side of Eq.(2-13), \bar{z}_k , corresponds to \bar{z}_1 in Eq.(2-3), and the second term, \bar{z}_{corr} , is the correction term. It can easily be seen that \bar{z}_1 in Eq.(2-13) is reduced to \bar{z}_k when $\tau(k_{-1} + k_{-2}) \gg 1$, whereas \bar{z}_1 is reduced to $\bar{z}_d / (K_\alpha + K_\beta)$ when $\tau(k_{-1} + k_{-2})$ becomes zero. These are quite reasonable conclusions. Recently Nishihara and Matsuda⁴⁴⁾ have carried out more rigorous mathematical analysis of the problem by use of an electronic computer. Preliminary examination showed that the errors resulted from using Eqs.(2-13) to (2-16) did not exceed 8 % for

$(\tau k)^{1/2} < 2$ under the conditions (2-17) and (2-18).

As the mutarotation takes place in the bulk of the solution, the bulk concentrations of α - and β -pyranose, C_α and C_β , change with time. For example, if we dissolve α -pyranose form in a solution at time $t = 0$, C_α decreases with time, whereas C_β increases, in accordance with the following equations:⁸⁾

$$C_\alpha = (C_\alpha)_{t \rightarrow \infty} (1 + K \exp(-k_0 t)) \quad (2-19)$$

$$C_\beta = (C_\beta)_{t \rightarrow \infty} (1 - \exp(-k_0 t)) \quad (2-20)$$

where:

$$k_0 = \frac{k_1 k_{-2} + k_{-1} k_2}{k_{-1} + k_{-2}} \quad (2-21)$$

$$K = \frac{(C_\beta)_{t \rightarrow \infty}}{(C_\alpha)_{t \rightarrow \infty}} = \frac{K_\beta}{K_\alpha} \quad (2-22)$$

and:

$$C_\alpha + C_\beta = C_{total} = (C_\alpha)_{t=0} \quad (2-23)$$

Here the concentration of intermediate γ -form is assumed to be negligibly small. Thus, the polarographic current which is given by Eq.(2-3) or Eq.(2-13), changes with time. The combination of Eqs.(2-19) to (2-23) with Eq.(2-13) to analyze the polarographic current, that was recorded as a function of the time, makes it possible to compute the four rate constants, k_1 , k_2 , k_{-1} , and k_{-2} , in Eq.(2-2).

If we dissolve β -pyranose in a solution at time $t = 0$, similar equations to Eqs.(2-19) to (2-23) can be obtained, in which the subscripts α and β should be replaced by β and α , respectively.

II-2. DETERMINATION OF MUTAROTATION RATE CONSTANTS OF MONOSACCHARIDES

The mutarotation rate constants, k_1 , k_2 , k_{-1} , and k_{-2} , have been determined only for the case of α -D-glucose in a neutral phosphate buffer solution.⁸⁾ In this study the author have extended the measurement over several monosaccharides and applied the modified equations to determine the individual rate constants of mutarotation in neutral and basic buffer solutions. Polarographic behavior of β -D-glucose has been studied by Tsukamoto,⁷⁾ but his approach is rather different from the present one.

EXPERIMENTAL

Materials The α -D-glucose ($[\alpha]_D^{25} + 112$) and β -D-glucose ($[\alpha]_D^{25} + 24$) were gift from Tokai Togyo Co. Ltd. The α -D-galactose ($[\alpha]_D^{25} + 150$) and β -D-galactose ($[\alpha]_D^{25} + 53$) were recrystallized from hot ethanol and cold ethanol, respectively. The α -D-xylose was recrystallized from water ($[\alpha]_D^{25} + 94$).⁴⁶⁾ Values of the specific rotation of these monosaccharides, except β -D-glucose, were identical with reported values.²⁵⁾ The sample of β -D-glucose had contained 6 % of α -form (calculated from the data of the specific rotation.²⁵⁾). All the other chemicals (reagent grade) were obtained commercially. The buffer components were NaH_2PO_4 - Na_2HPO_4 and NH_3 - NH_4Cl . Potassium chloride was used to adjust the ionic strength (usually 0.5).

Apparatus Polaro-recorder, polarimeter and pH meter used were described in Chapter I. An electrolysis cell (20 ml in capacity) was used; it was connected to SCE by means of an agar-gelatine bridge containing potassium chloride. All experiments were carried out in a water thermostat controlled at $25 \pm 0.05^\circ\text{C}$.

Methods A supporting electrolyte solution (15 ml) of desired

composition was freed of oxygen by passing a nitrogen stream through the solution. First, the residual current was recorded by means of the polarograph. Second, after the potential of the dropping mercury electrode had been set at -1.75 V vs. SCE, a weighed amount of α -pyranose or β -pyranose was dissolved in the deoxygenated electrolyte solution and the passing of the nitrogen stream through the solution was continued for about 30 seconds to complete the dissolution of the monosaccharide. Then, the current intensity at -1.75 V vs. SCE was recorded as a function of the time.

The recording of the current was continued until the current intensity reached a constant value. The nitrogen stream was passed over the solution during the measurement. Finally, after the mutarotation equilibrium had been reached, a whole polarographic wave of the monosaccharide was recorded. The current intensity was corrected for the residual current.

RESULTS AND DISCUSSION

When α -pyranose was dissolved in the electrolyte buffer solution the polarographic limiting current decreased with time. While the limiting current increased with time when β -pyranose was dissolved. These results suggest that the conversion velocity of α -pyranose to γ -form is larger than that of β -pyranose to γ -form. After the equilibrium had been reached, the value of the limiting current attained with α -pyranose agreed with that attained with β -pyranose within experimental errors. As a typical example, Fig. II-1 shows the time dependence of the limiting current of β -D-galactose in 0.1M ammonia buffer solution of pH 9.14; the time dependence of the limiting current of α -D-galactose in the same buffer solution is shown in Fig. II-2. Guggenheim plot of the $\bar{i}_1 - t$ relationship gave a straight line. The slope of this line gives the overall mutarotation rate constant, k_o , defined by Eq.(2-21). An example of this plot is shown in Fig. II-3.

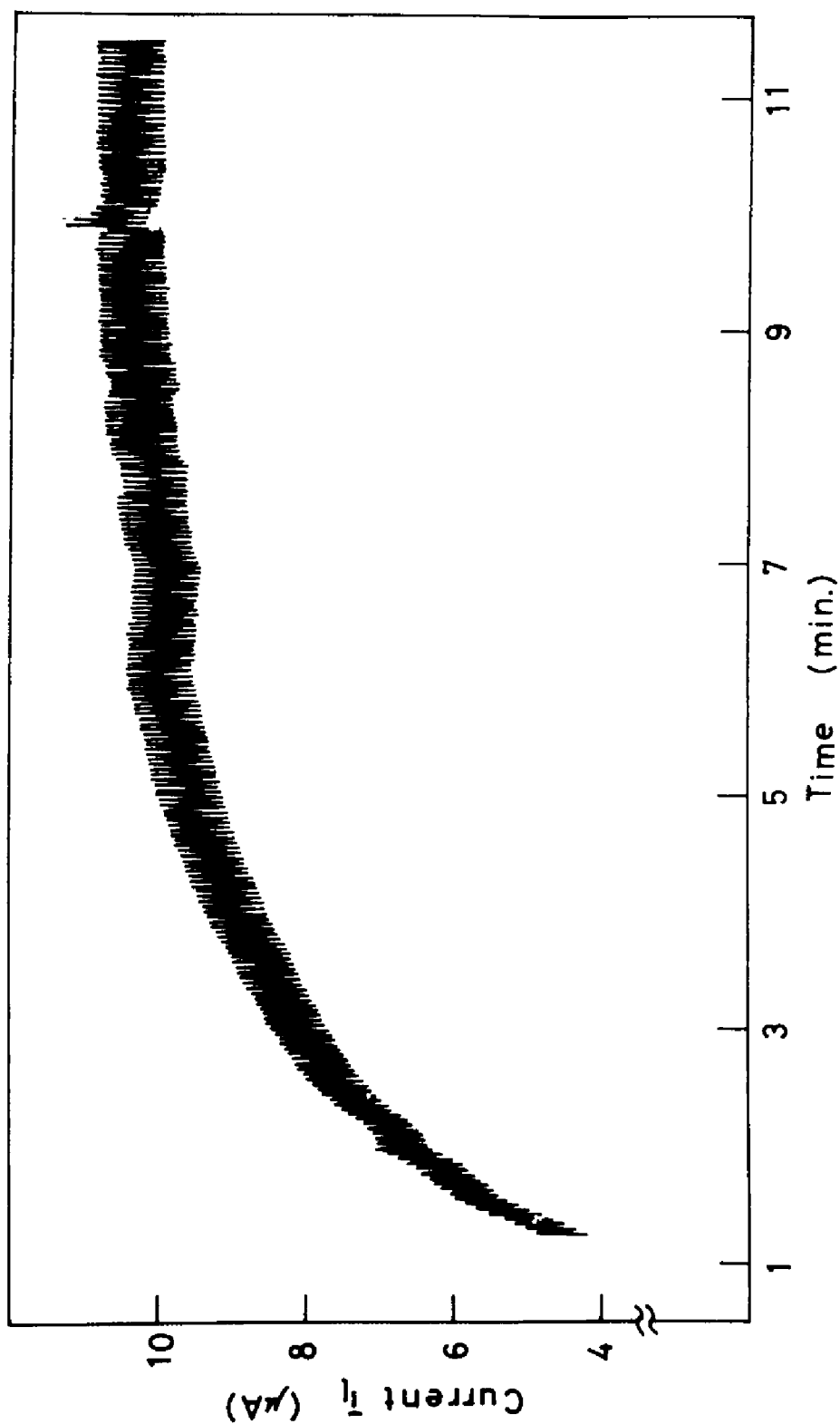


Fig.II-1 The limiting current *vs.* time curve when β -D-galactose is dissolved at time $t=0$ in 0.1M ammonia buffer solution of pH 9.14 and of ionic strength 0.5.

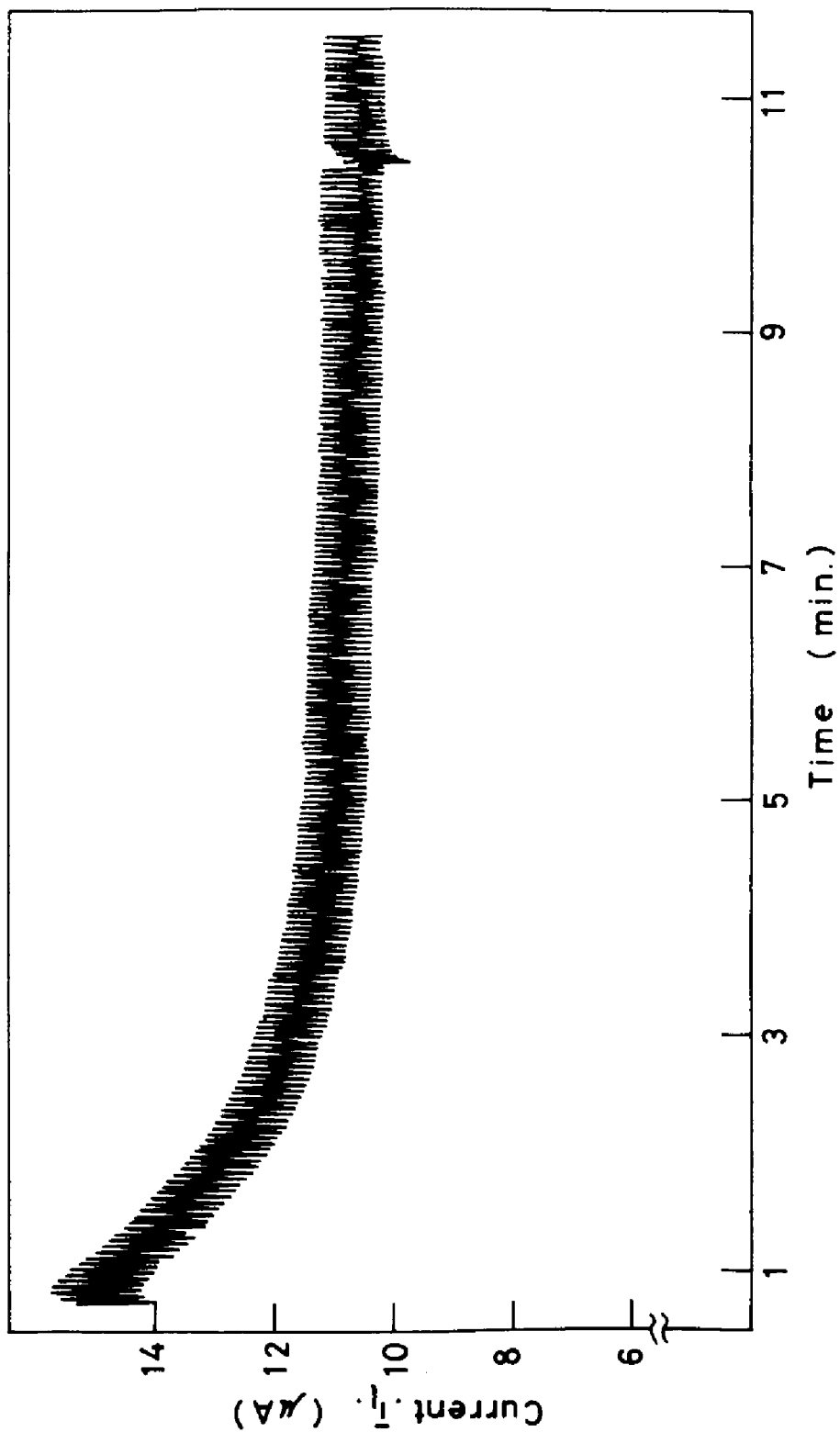


Fig.II-2 The limiting current i_L vs. time curve when α -D-galactose is dissolved at time $t=0$ in 0.1M ammonia buffer solution of pH 9.14 and of ionic strength 0.5.

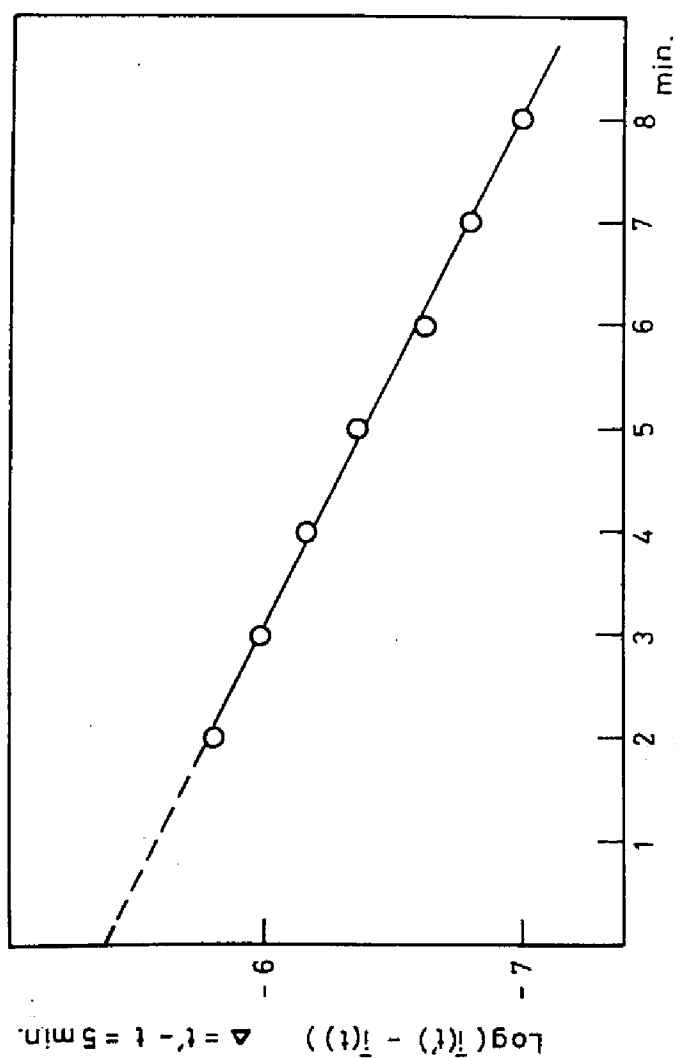


Fig. II-3 Analysis of current *vs.* time curve when β -D-galactose is dissolved at time $t=0$ in 0.1 M ammonia buffer solution of pH 9.14 and of ionic strength 0.5.

The individual rate constants, k_1 , k_2 , k_{-1} , and k_{-2} , were computed by applying the theoretical equations derived above. In applying Eqs. (2-13) to (2-15) and (2-4), a successive approximation was carried out by use of an electronic computer, FACOM 230-60 (Kyoto University). The diffusion coefficient, D , was assumed to be $6.16 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$ for D-glucose, $6.38 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$ for D-galactose, and $6.50 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$ for D-xylose,⁴⁵⁾ and the equilibrium constant K (defined by Eq. (2-22)) was assumed to be 1.74 for D-glucose, 2.38 for D-galactose, and 1.87 for D-xylose.¹⁴⁾

Table II-1 shows the values of the rate constants determined by analysing the time dependence of the polarographic limiting currents observed when α -pyranose or β -pyranose was dissolved. As expected from the reaction scheme (2-2), the values of the rate constants determined separately from the time course α - to β -pyranose and β - to α -pyranose were coincident within the experimental error. Note that the values of the rate constants for D-galactose may contain an error of a few percent because of the existence of the furanose form of a few percent.⁴⁷⁾

TABLE II-1. THE OVERALL AND INDIVIDUAL MUTAROTAION RATE CONSTANTS DETERMINED FROM α -PYRANOSE AND β -PYRANOSE AT 25°C

Sugar	Determined from the time course of		$k_0 \times 10^3$	$k_1 \times 10^3$	$k_2 \times 10^3$ (sec ⁻¹)	k_{-1}	k_{-2}
D-glu.	α	$\rightarrow \beta$	2.7	4.1	1.7	36	26
	β	$\rightarrow \alpha$	2.8	6.9	1.4	95	34
(0.07 M phosphate buffer pH 6.99)							
D-gal.	α	$\rightarrow \beta$	2.4	4.0	1.4	0.7	0.5
	β	$\rightarrow \alpha$	2.2	2.9	1.5	0.5	0.5
(0.10 M ammonia buffer pH 8.12)							

Table II-2 shows the values of the overall rate constant and the individual rate constants for D-glucose, D-galactose, and D-xylose. In both the neutral phosphate and the basic ammonia buffer solutions, the values of k_0 and the forward rate constants, k_1 and k_2 , increased in the order of D-glucose < D-galactose < D-xylose. This order is in agreement with the increasing order of the overall mutarotation rate constants of these sugars measured in water by the polarimetric method.¹⁴⁾ Backward rate constants, k_{-1} and k_{-2} , increased in the order of D-galactose < D-xylose < D-glucose. The ratio of the concentration of the intermediate γ -form to the concentration of the pyranose form, R , was calculated according to the relationship:

$$R = \frac{C_v}{(C_\alpha + C_\beta)} = \frac{k_1 k_2}{k_{-1} k_2 + k_1 k_{-2}} \quad (2-24)$$

TABLE II-2. THE OVERALL AND THE INDIVIDUAL MUTAROTATION RATE CONSTANTS OF MONOSACCHARIDES AT 25°C

Sugar	Concn. of buffer and pH	$k_0 \times 10^3$	$k_1 \times 10^3$	$k_2 \times 10^3$ (sec ⁻¹)	k_{-1}	k_{-2}
α -D-glu.	0.1 M phosphate pH 6.99	4.4	6.7	2.3	109	76
α -D-gal.	"	4.5	7.3	2.9	3.5	3.1
α -D-xyl.	"	17	41	8.6	61	23
α -D-glu.	0.1 M ammonia pH 8.60	1.9	3.2	1.1	3.3	2.0
α -D-gal.	"	4.4	6.9	2.6	0.5	0.4
α -D-xyl.	"	8.5	16	4.6	1.6	1.0

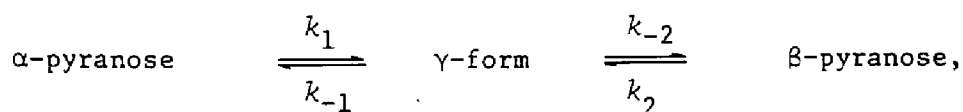
As shown in Table II-3, the values of R increased in the order of D-glucose < D-xylose < D-galactose. In the ammonia buffer solution, R for each monosaccharide was about ten times larger than that in the phosphate buffer solution. In addition, R for each monosaccharide depended slightly on the concentration of the buffer component. The data in Table II-3 are the averaged values of the results in several buffer concentrations. A similar dependence of R on the buffer concentration has also been reported by previous workers for the case of α -D-glucose in the phosphate buffer solution.⁸⁾ These results are interesting in view of the mechanism of an acid-base catalyzed mutarotation.

TABLE II-3. THE RATIO, R , OF THE CONCENTRATION OF THE REDUCIBLE INTERMEDIATE γ -FORM TO THE CONCENTRATION OF THE PYRANOSE FORM AT 25°C

Sugar	R (%)	
	Phosphate buffer of pH 6-7	Ammonia buffer of pH 8-9
D-glu.	3×10^{-3}	4×10^{-2}
D-gal.	5×10^{-2}	4×10^{-1}
D-xyl.	3×10^{-2}	3×10^{-1}

SUMMARY

Wiesner's equations, relating the kinetic current with four rate constants of the equation:



were modified so that the equation can be applied for the case of the

smaller values of the rate constants, where Matsuda-Koutecky's equation of the kinetic current cannot exactly be applied.

By use of these modified equations, the individual rate constants and the overall rate constant were calculated for α - and β -D-glucose, α - and β -D-galactose, and α -D-xylose in neutral phosphate and basic ammonia buffer solutions.

The ratio of the concentration of the intermediate γ -form to the concentration of the pyranose form was calculated.

CHAPTER III. ACID-BASE CATALYZED MUTAROTATION OF α -D-XYLOSE

The mutarotation reaction of monosaccharides is one of the most typical examples of the general acid-base catalytic reaction.^{15,16)} However, the mechanism of this reaction has not been made sufficiently clear. One of the reasons of this ambiguity is that all of the methods, except polarography, can be used to determine only the overall rate constant, k_o , of the mutarotation assumed as:

α -pyranose $\xrightleftharpoons[k_B]{k_A}$ β -pyranose. On the other hand, we can detect the

reducible intermediate γ -form of the mutarotation reaction by the polarographic method. Thus, it becomes possible to calculate the individual mutarotation rate constants, where the reaction scheme:

α -pyranose $\xrightleftharpoons[k_{-1}]{k_1}$ γ -form $\xrightleftharpoons[k_2]{k_{-2}}$ β -pyranose is assumed (Chapter II).

It is shown in Chapter II that the overall and individual rate constants of monosaccharides depend on the buffer components of the experimental solutions. This dependence of the rate constants on the buffer component is important in elucidating the reaction mechanism of the acid-base catalyzed mutarotation. This chapter is concerned with detailed studies on the effect of the various buffer components on the rate constants of mutarotation of α -D-xylose. The temperature effects on the reaction are also described.

EXPERIMENTAL

Materials The α -D-xylose was used because of the simpleness of its structure and the easiness of its purification. It was recrystallized as described in the previous chapter. All other chemicals (reagent grade) were obtained commercially. Solutions were prepared with bidistilled water. The buffer components were NaH_2PO_4 - Na_2HPO_4 , NH_3 - NH_4Cl , Tris(hydroxymethyl)amino methane (TRIS),

Tris(hydroxymethyl) methyl glycine (TRICIN), and NN-bis(2-hydroxyethyl) glycine (BICIN). Potassium chloride was used to adjust the ionic strength to desired values.

Apparatus and Methods Apparatus, procedures for polarographic measurements and the computation method of individual rate constants of the mutarotation reaction given by Eq.(2-2) were the same as described in Chapter II. For studying the temperature effects, the measurements were carried out in phosphate buffers at 20°C, 25°C, and 30°C±0.05°C.

RESULTS

General polarographic behavior of D-xylose The general polarographic behavior of the α - β -equilibrated mixture of D-xylose in an ammonia buffer solution is discussed in Chapter I. A few examples of the experimental results on the dependence of the limiting current, \bar{i}_1 , on the height of the mercury reservoir, h , are shown in Table III-1. The limiting current was inclined to increase slightly with the increasing height of the mercury reservoir. An analysis of the current-time (i - t) curve for a single drop (Fig.III-1) has shown that the slope of $\log i$ vs. $\log t$ plot is 1.8/3, which is slightly smaller than the theoretical value, 2/3, which is expected for a purely kinetic current defined by Eq.(2-3). These results support the idea that the correction term, \bar{i}_{corr} , in Eq. (2-13) should not be neglected in interpreting the kinetic current produced by D-xylose.

The limiting current is proportional to the D-xylose concentration in various supporting electrolyte solutions, as is shown by Curves A, B, and D, in Fig. III-2, but at a very low concentration of the buffer component a deviation from the linearity was observed, as is exemplified by Curve C in Fig.III-2. As described in Chapter I, in the basic solution the limiting current increased exponentially

TABLE III-1. DEPENDENCE OF THE LIMITING CURRENT, \bar{i}_1 , ON THE HEIGHT OF MERCURY RESERVOIR, h , AT 25°C

h (cm)	\bar{i}_1 (cm)		
	Phosphate buffer	TRIS buffer	TRIS buffer
	0.05 M, pH 6.99	0.05 M, pH 8.04	0.20 M, pH 8.04
62.4	2.36	4.40	4.81
72.4	2.47	4.52	5.01
82.4	2.45	4.60	5.02
c.f. \bar{i}_1/\bar{i}_d	2.96×10^{-3}	5.67×10^{-3}	6.28×10^{-3}

D-Xylose: 0.066 M, Current sensitivity: 0.4 μ A/cm

with the pH; and the limiting current decreased with a decrease in the concentration of the buffer component at a given pH. On the contrary, in the neutral phosphate buffer solution, the limiting current first decreased with a decrease in the concentration of the buffer component, but at a lower concentration (0.005 M) it began to increase and finally reached an unusually large value in an unbuffered solution. A typical example is shown in Table III-2. The last result might be explained by the autocatalytic effect of hydroxyl ion produced in company with the electroreduction of D-xylose at the electrode surface. A similar phenomenon has been reported by Brdicka and coworkers^{48,49)} for the electro-reduction of formaldehyde at the dropping mercury electrode in an unbuffered solution. The polarographic behavior of monosaccharides in unbuffered solutions will be discussed in Chapter V.

Determination of mutarotation rate constants of α -D-xylose

The composition and pH's of the buffer solution in which the mutarotation rate constants were determined are summarized in Table III-3.

TABLE III-2. THE LIMITING CURRENT, \bar{i}_1 , OF D-XYLOSE WITH VARIOUS BUFFER CONCENTRATIONS AT CONSTANT pH

Concn. of phosphate buffer at pH 6.99 (M)	\bar{i}_1 (μ A)	Concn. of ammonia buffer at pH 9.40 (M)	\bar{i}_1 (μ A)
0.100	1.06	0.533	4.01
0.050	0.92	0.268	3.44
0.010	0.68	0.133	3.03
0.005	0.67	0.067	2.78
0.001	1.22	0.033	2.68
none	9.68		

TABLE III-3. BUFFER SOLUTIONS

Buffer	Base component	pK _A	Concn. (M)	pH
Phosphate	HPO ₄ ²⁻	7.2	0.03 - 0.10	6.12 - 6.99
TRICIN	(CH ₂ OH) ₃ -CNH -OOCCH ₂	8.1	0.02 - 0.20	7.88 - 8.42
TRIS	(CH ₂ OH) ₃ -CNH ₂	8.2	0.02 - 0.20	8.04 - 8.42
BICIN	(C ₂ H ₄ OH) ₂ -N -OOCCH ₂	8.3	0.02 - 0.15	8.10 - 8.68
Ammonia	NH ₃	9.2	0.05 - 0.50	8.16 - 8.60

D-Xylose: 0.132-0.066 M, Temp.: 25°C, Ionic strength: 0.5.

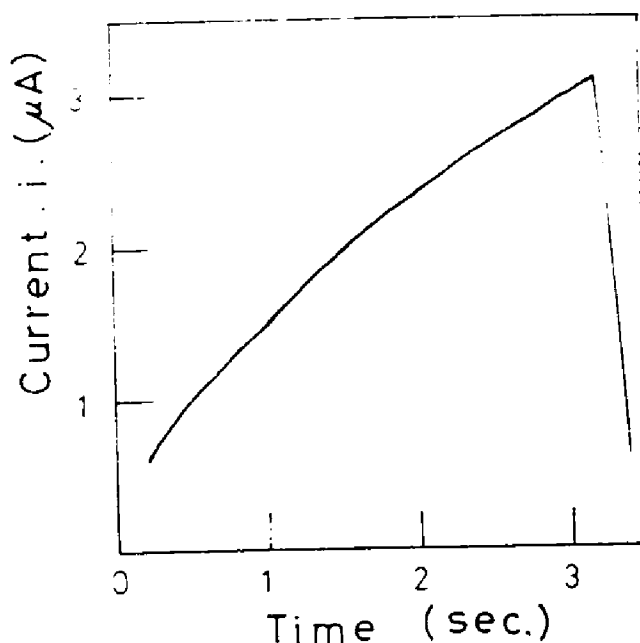


Fig. III-1 Instantaneous current vs. time curve for a single drop of mercury of D-xylose (0.066 M) in ammonia buffer solution of pH 8.12. Recorded at -1.75 Volt vs. SCE.

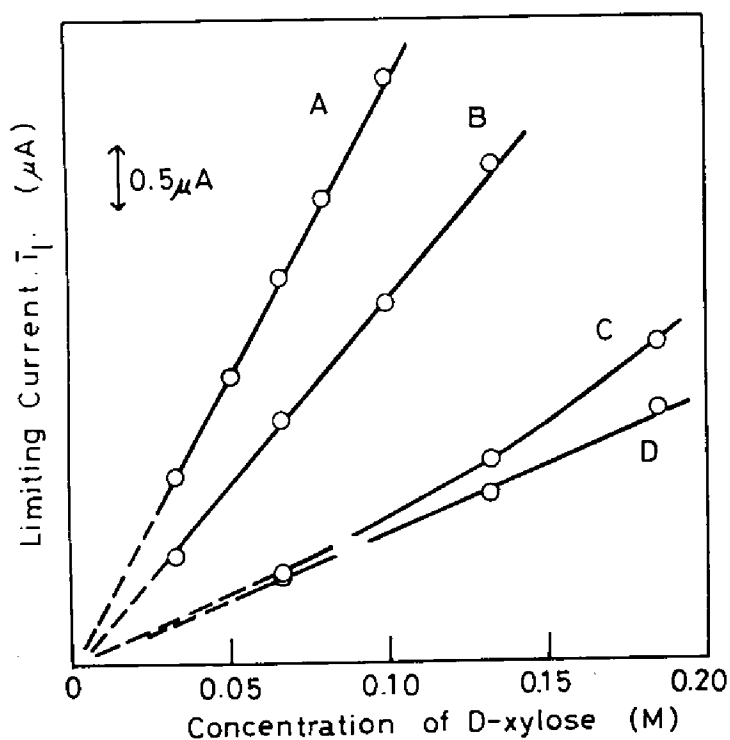


Fig. III-2 Relationship between the limiting current and the concentration of D-xylose at temp. 25°C. A: Ammonia buffer 0.100M, pH 8.79, B: TRIS buffer 0.020M, pH 8.25, C: Phosphate buffer 0.005M, pH 6.99, D: Phosphate buffer 0.070M, pH 6.12.

These conditions have been selected to avoid complexities arising from too high a basicity or too low a buffer concentration, as has been described above. Too high a concentration of buffer components resulted in an increase in the final ascending of the base current and made accurate measurement of the limiting current difficult.

The analyzing procedure of the experimental results has been described in the previous chapter. Examples of the four individual rate constants and the overall rate constant defined by Eqs.(2-2) and (2-21), are shown in Table III-4. The backward or ring-closing rate constants, k_{-1} , and k_{-2} , which could be determined in general only with a poor precision, were not always a linear function of the concentrations of buffer components. Accordingly, a detailed analysis of the rate constants was made with the forward or ring-opening rate constants, k_1 , and k_2 , and the overall rate constant, k_0 .

At a constant pH, the rate constants, k_0 , k_1 , and k_2 , increased linearly to the buffer concentration. Results are shown in Fig. III-3 to III-7. Generally, the rate constant of a catalyzed reaction can be expressed as follows:¹⁶⁾

$$k_i = k_{i,w} + k_{i,OH}(OH^-) + k_{i,A}(A) + k_{i,B}(B) \quad (3-1)$$

$i = 0, 1, \text{ and } 2,$

where $k_{i,w}$ represents the catalytic constant of the solvent molecule (water in this case), $k_{i,OH}$, $k_{i,A}$, and $k_{i,B}$ the catalytic coefficients of the catalysts indicated by the subscripts, and the symbols in brackets the concentrations (activities) of the catalysts, OH^- , A, and B, being the hydroxyl ion, acid and base of buffer components. In this expression, the concentration of hydrogen ion is neglected because the measurements were made in neutral or basic solutions. The contribution of the D-xylose anion is also neglected. The significance of this last approximation will be discussed later.

TABLE III-4. THE OVERALL AND THE INDIVIDUAL RATE CONSTANTS OF
D-XYLOSE IN VARIOUS BUFFER CONCENTRATIONS AT 25°C

Buffer	Rate constant (sec ⁻¹)					R* (%)
	$k_0 \times 10^3$	$k_1 \times 10^3$	$k_2 \times 10^3$	k_{-1}	k_{-2}	
Phosphate 0.05M, pH6.99	9.1	16.2	5.0	15.2	8.6	3.6×10^{-2}
TRICIN 0.10M, pH8.22	5.3	8.1	3.2	0.9	0.7	3.0×10^{-1}
TRIS 0.10M, pH8.27	6.5	9.9	3.8	1.3	0.9	2.7×10^{-1}
BICIN 0.10M, pH8.29	3.4	4.5	2.2	0.4	0.4	3.7×10^{-1}
Ammonia 0.10M, pH8.60	8.5	16.0	4.6	1.6	1.0	3.2×10^{-1}

*R: The ratio of the concentration of the reducible intermediate γ -form to the concentration of the pyranose form.

When the rate constant is plotted against the sum of the concentrations of the buffer components, $C_{buff} = (A) + (B)$, at a given pH, a straight line will be obtained; the slope of this line is $(k_{i,A} + k_{i,B})/(1 + N)$, N being $(A)/(B)$, and the intercept on the k_i -axis is $k_{i,w} + k_{i,OH}(OH^-)$. A plot of this intercept against (OH^-) will give a straight line, from which $k_{i,OH}$ (slope) and $k_{i,w}$ (intercept) can be obtained. Fig.III-3 shows a plot of k_i vs. C_{buff} for phosphate buffer solutions of pH 6.12, 6.59, and 6.99. Three straight lines with different slopes are obtained, but their intercepts on the k_i -axis coincide within the limits of experimental error. These results suggest that $k_{i,OH}(OH^-)$ is negligible in comparison with $k_{i,w}$ in such neutral solutions. Fig.III-4 shows the same plot for the TRIS buffer; straight lines with different slopes and different intercepts on the k_i -axis are given for each solution

at a given pH. Similar results were obtained for all the other basic buffers (Fig.III-5-7). The intercept values thus obtained from the k_i vs. C_{buff} curves are plotted against (OH^-) in Fig.III-8. In accordance with theoretical considerations, all the data are distributed along the regression line, from which $k_{i,w}$ and $k_{i,OH}$ for individual as well as overall reaction rate are determined. The $k_{i,w}$ value obtained in this way was in good agreement with those obtained from the intercepts in Fig.III-3 (e.g. $k_{o,w} = 1.3 \times 10^{-3} \text{ sec}^{-1}$ from Fig.III-8 and $k_{o,w} = 1.4 \times 10^{-3} \text{ sec}^{-1}$ from Fig.III-3).

Fig.III-9 shows a plot of $k_i - (k_{i,w} + k_{i,OH}(OH^-))$ against (B) for the TRIS buffer. A straight line with a slope of $k_{i,B} + Nk_{i,A}$ can be expected from each for a given N . As may be seen from the figure, however, all the points lie on a single straight line irrespective of the value of N . This result suggests that $k_{i,P} \gg Nk_{i,A}$ i.e., $k_{i,A}$ is negligibly small compared with $k_{i,P}$ for the buffer components examined. Similar results were obtained for all the other basic buffers examined (Fig.III-10-12).

DISCUSSION

Effect of buffer component on the mutarotation rates of α -D-xylose The values of the catalytic rate coefficients are summarized in Table III-5. The coefficients, $k_{i,P}$'s for B = TRIS, TRICIN, and BICIN are those for the base components of the amine buffers. The water molecule is both an acid and a base, but for the mutarotation of D-glucose in a neutral solution, the basic catalytic function is supposed to predominate over the acid one.¹⁵⁾ For the mutarotation of D-xylose, the same argument is justified on the basis of the Brönsted plot (Fig.III-13). The HPO_4^{2-} and $H_2PO_4^-$ ions are also bifunctional, but in a neutral buffer solution the former ion functions as a base, and the latter as an acid. Accordingly, their catalytic activities as observed in a neutral solution, should in the first place be basic for HPO_4^{2-} and acidic

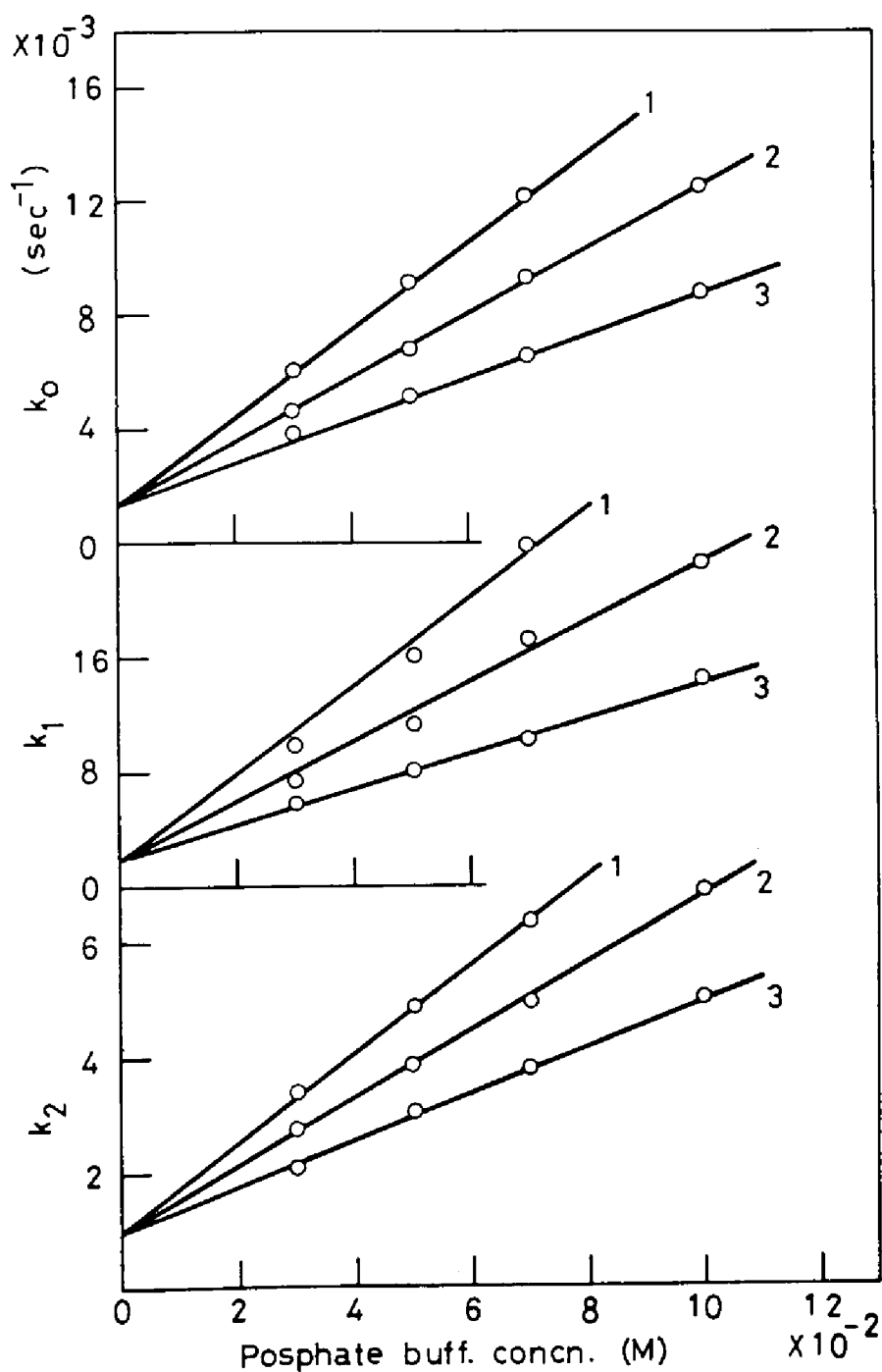


Fig.III-3 The rate constants as a function of the concentration of phosphate buffer at temp. 25°C; 1: pH 6.99, 2: pH 6.59, 3: pH 6.12.

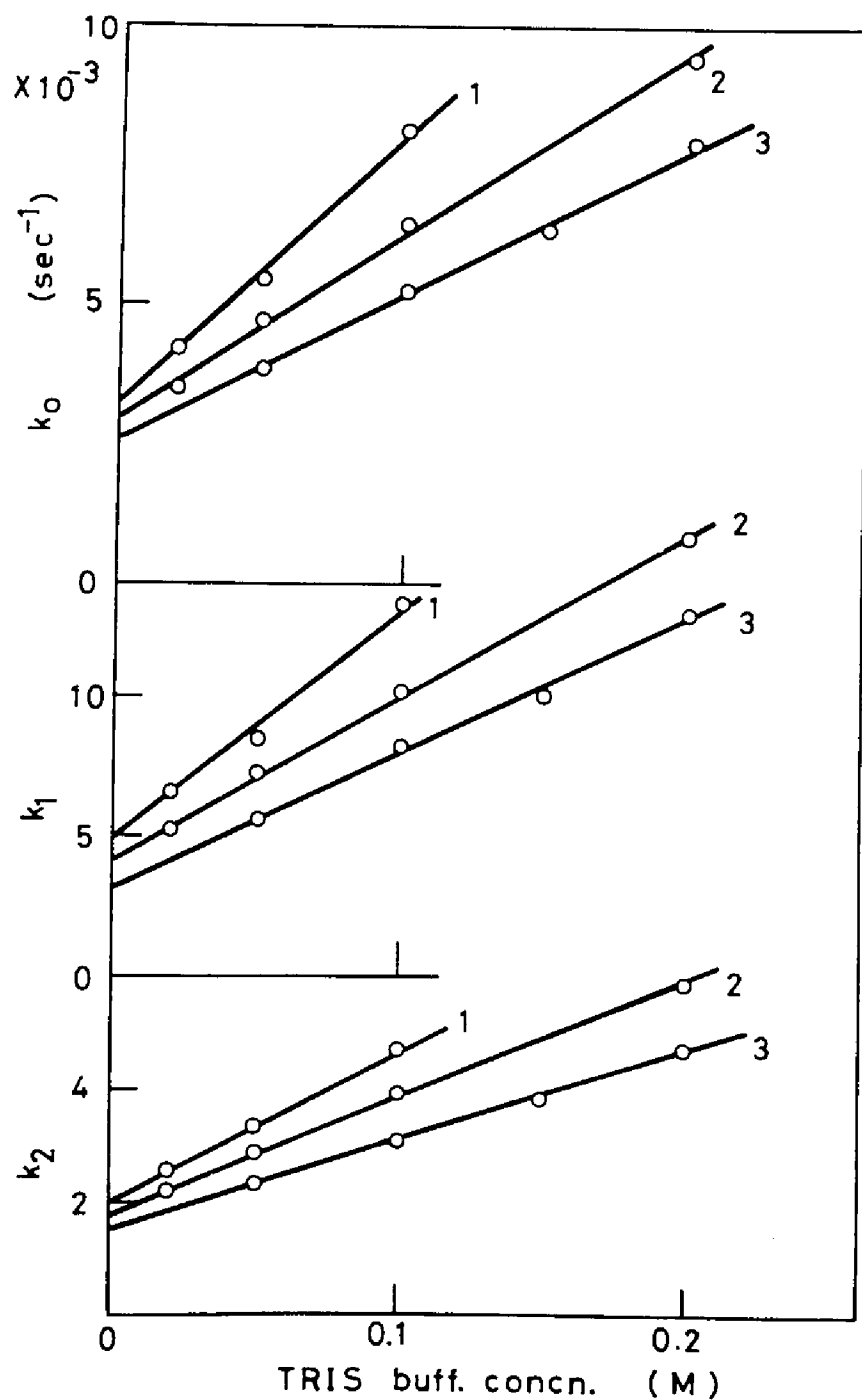


Fig.III-4 The rate constants as a function of the concentration of TRIS buffer at temp. 25°C; 1: pH 8.42, 2: pH 8.27, 3: pH 8.04.

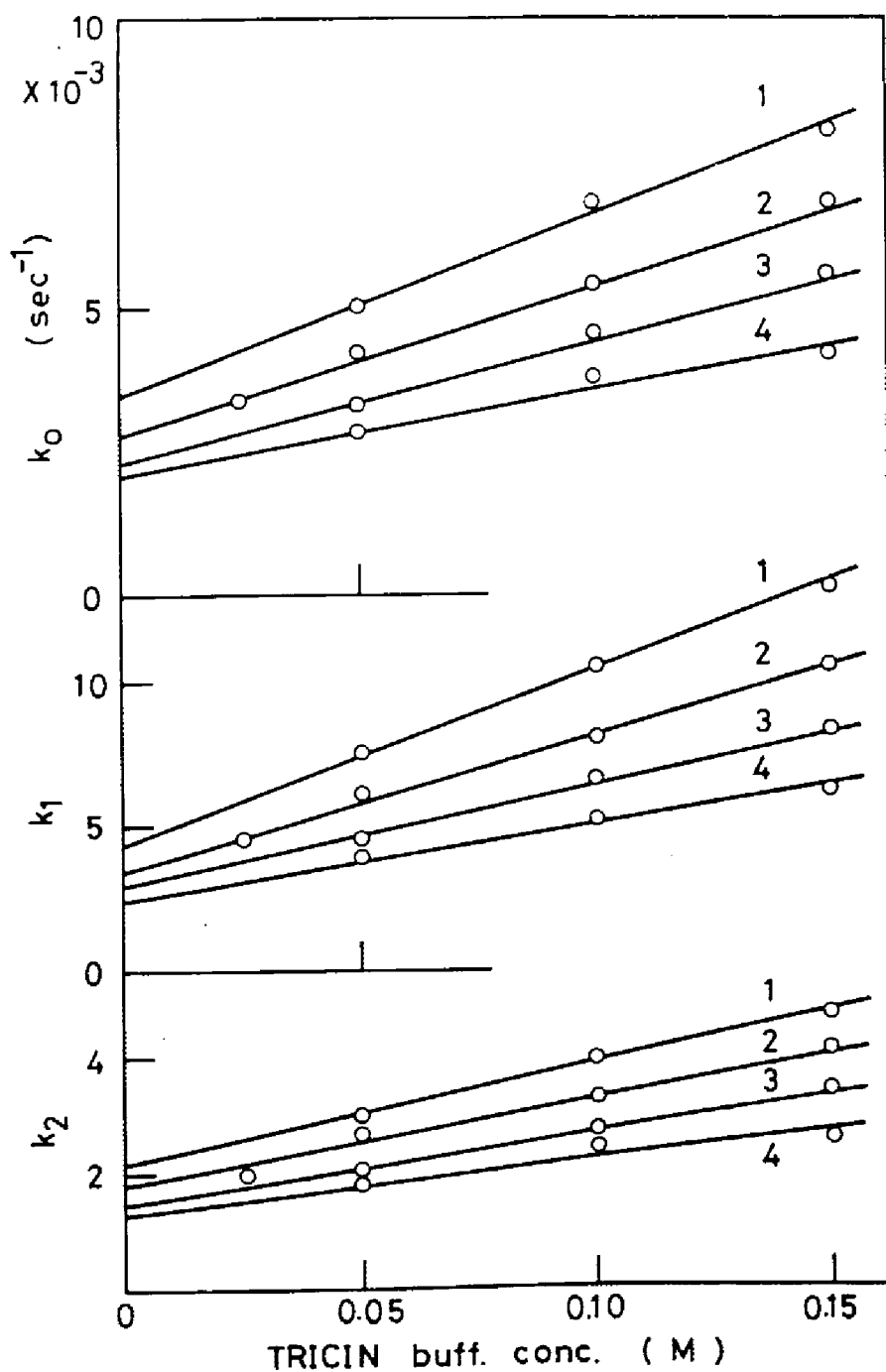


Fig.III-5 The rate constants as a function of the concentration of TRICIN buffer at temp. 25°C; 1: pH 8.42, 2: pH 8.22, 3: pH 8.05, 4: pH 7.88.

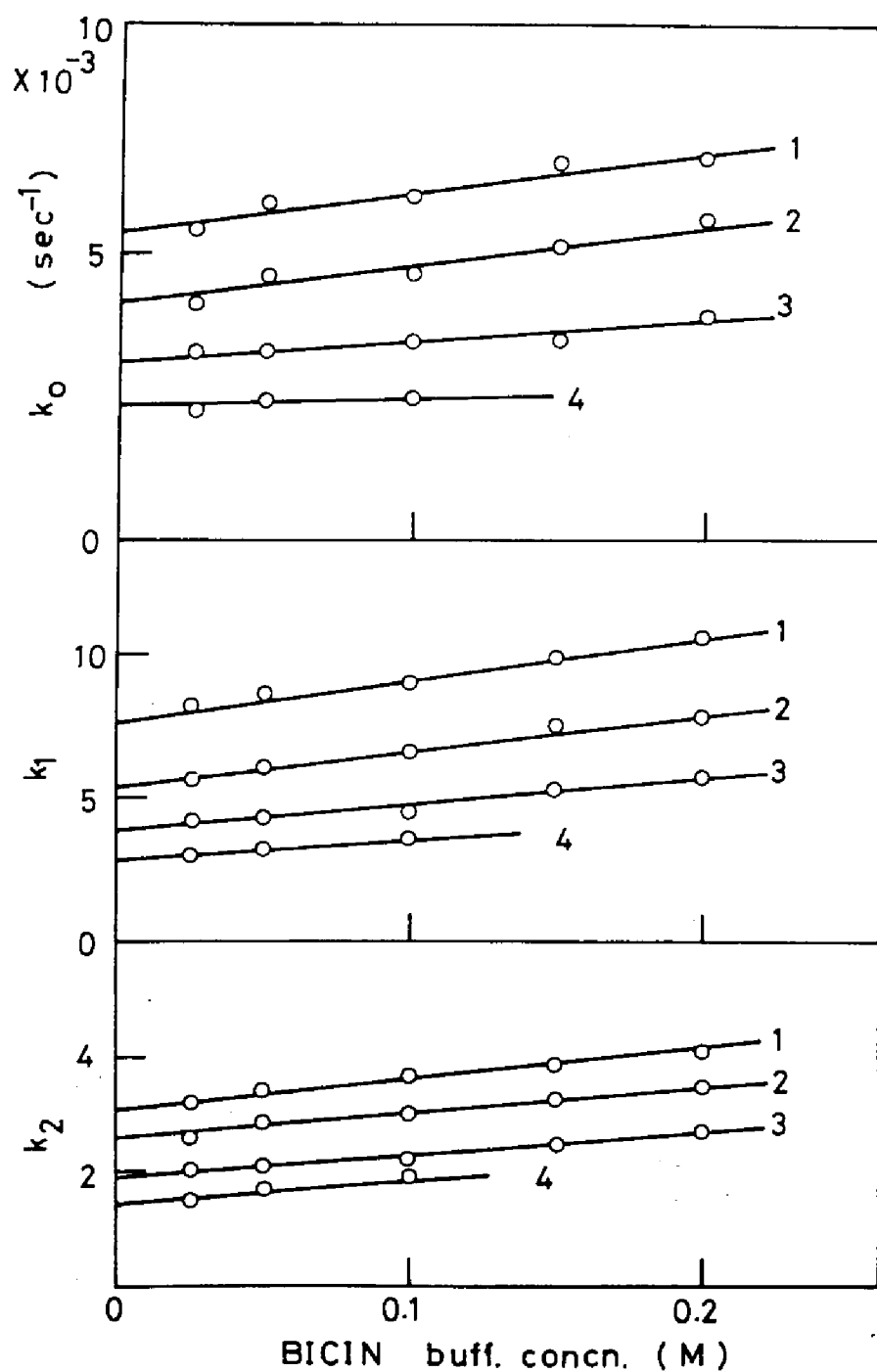


Fig.III-6 The rate constants as a function of the concentration of BICIN buffer at temp. 25°C; 1: pH 8.68, 2: pH 8.48, 3: pH 8.29, 4: pH 8.10.

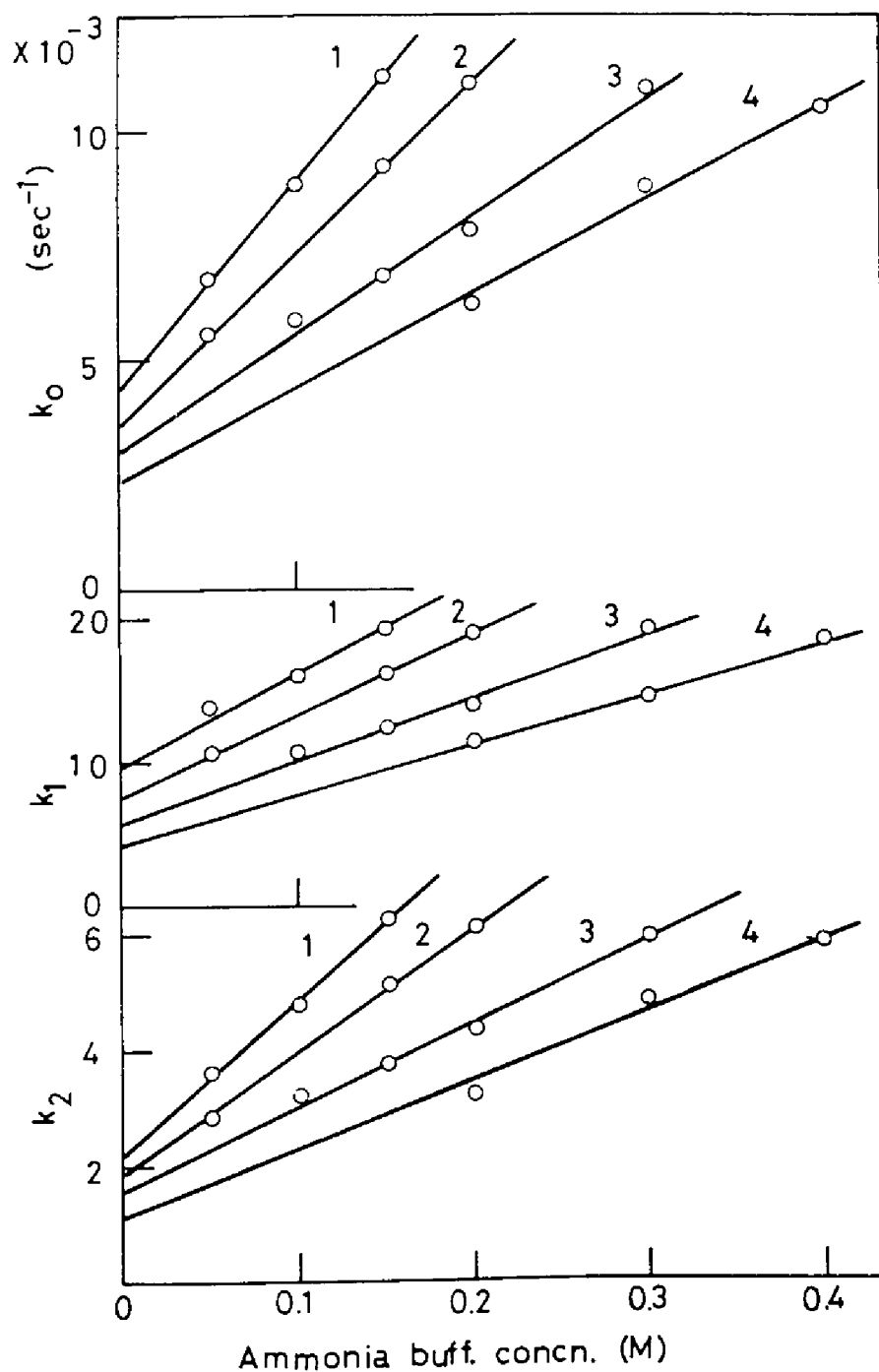


Fig.III-7 The rate constants as a function of the concentration of ammonia buffer at temp. 25°C; 1: pH 8.60, 2: pH 8.48, 3: pH 8.26, 4: pH 8.16.

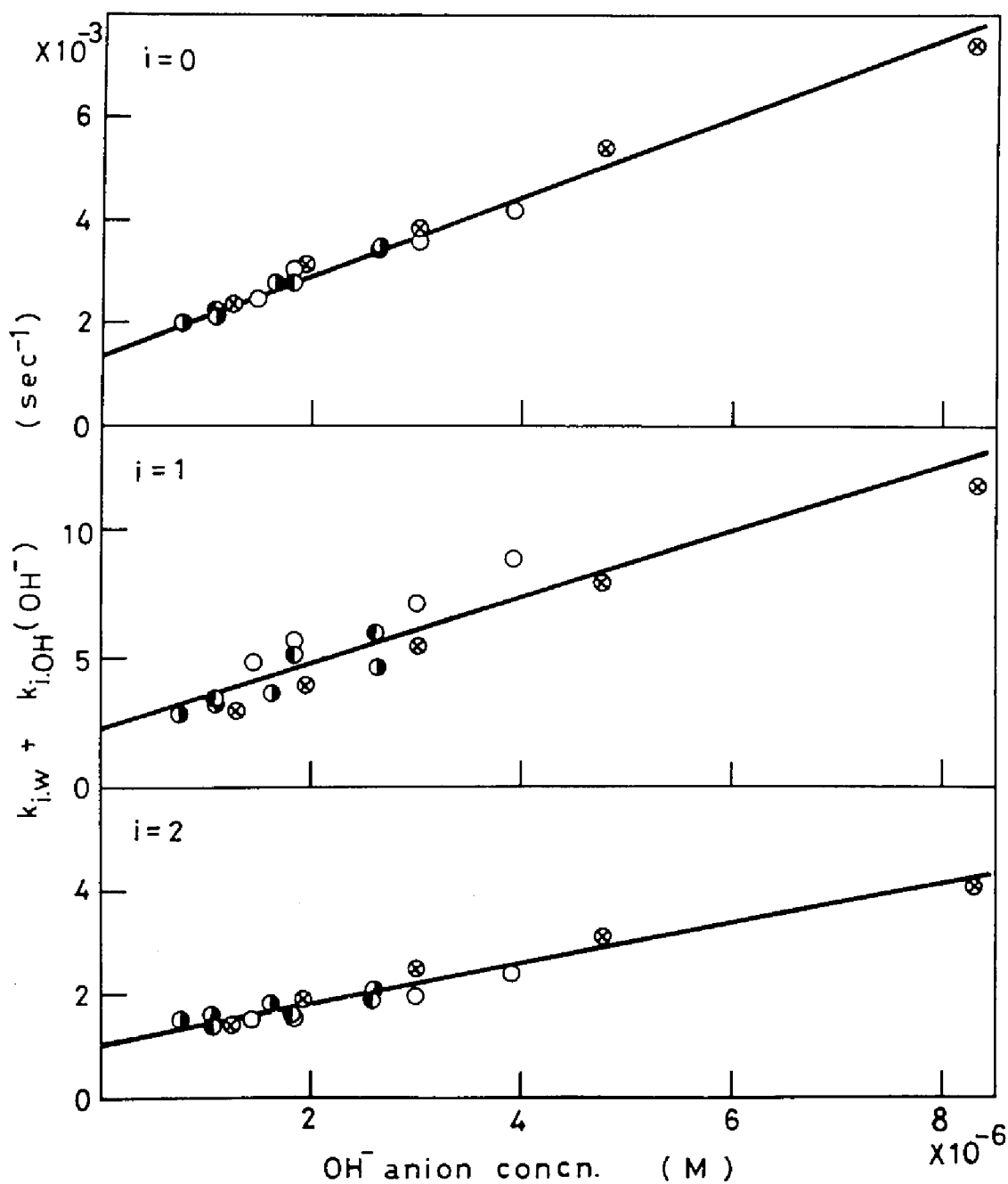


Fig.III-8 Plot of $k_{i,w} + k_{i,OH}(OH^-)$ against the concentration of hydroxyl ion. ○: Ammonia, ●: TRIS, ◐: TRICIN, ⊗: BICIN.

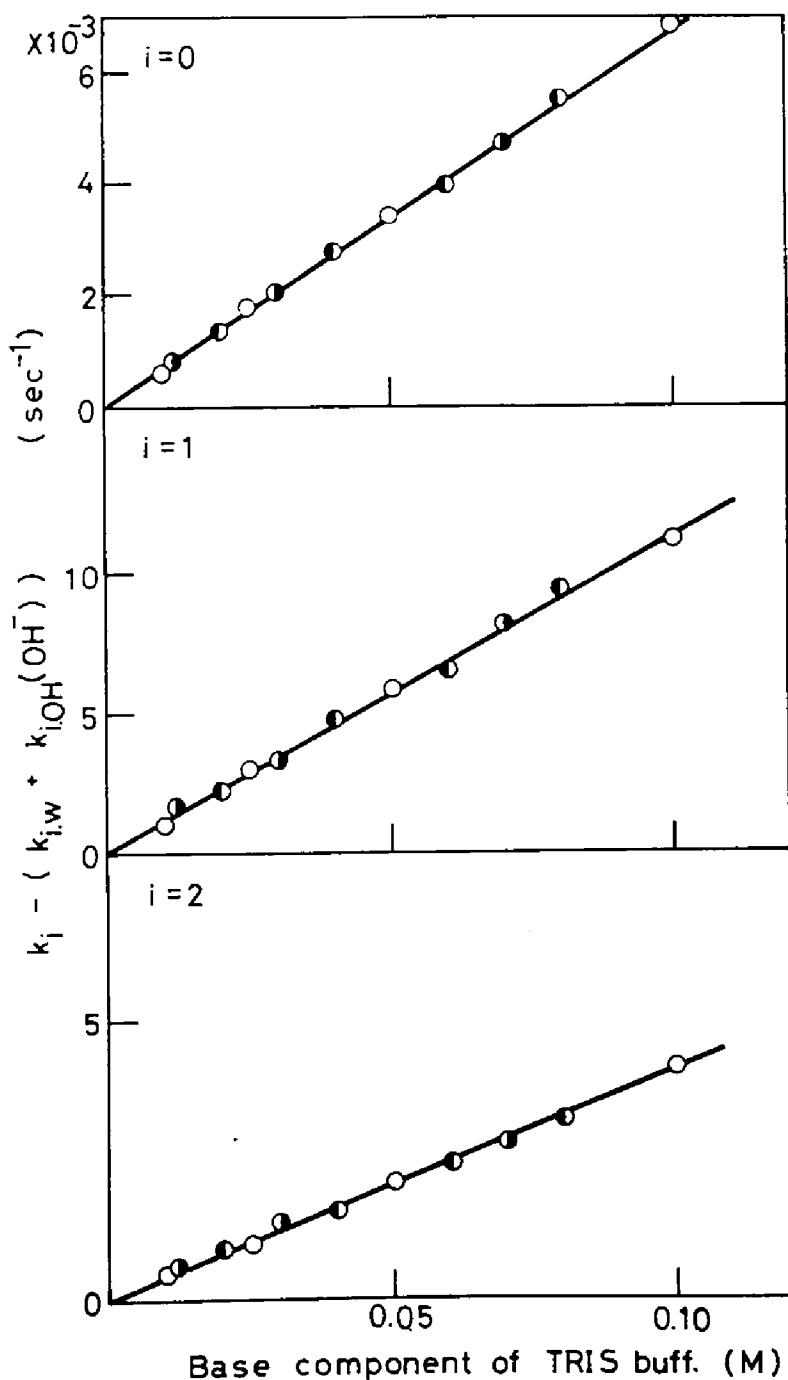
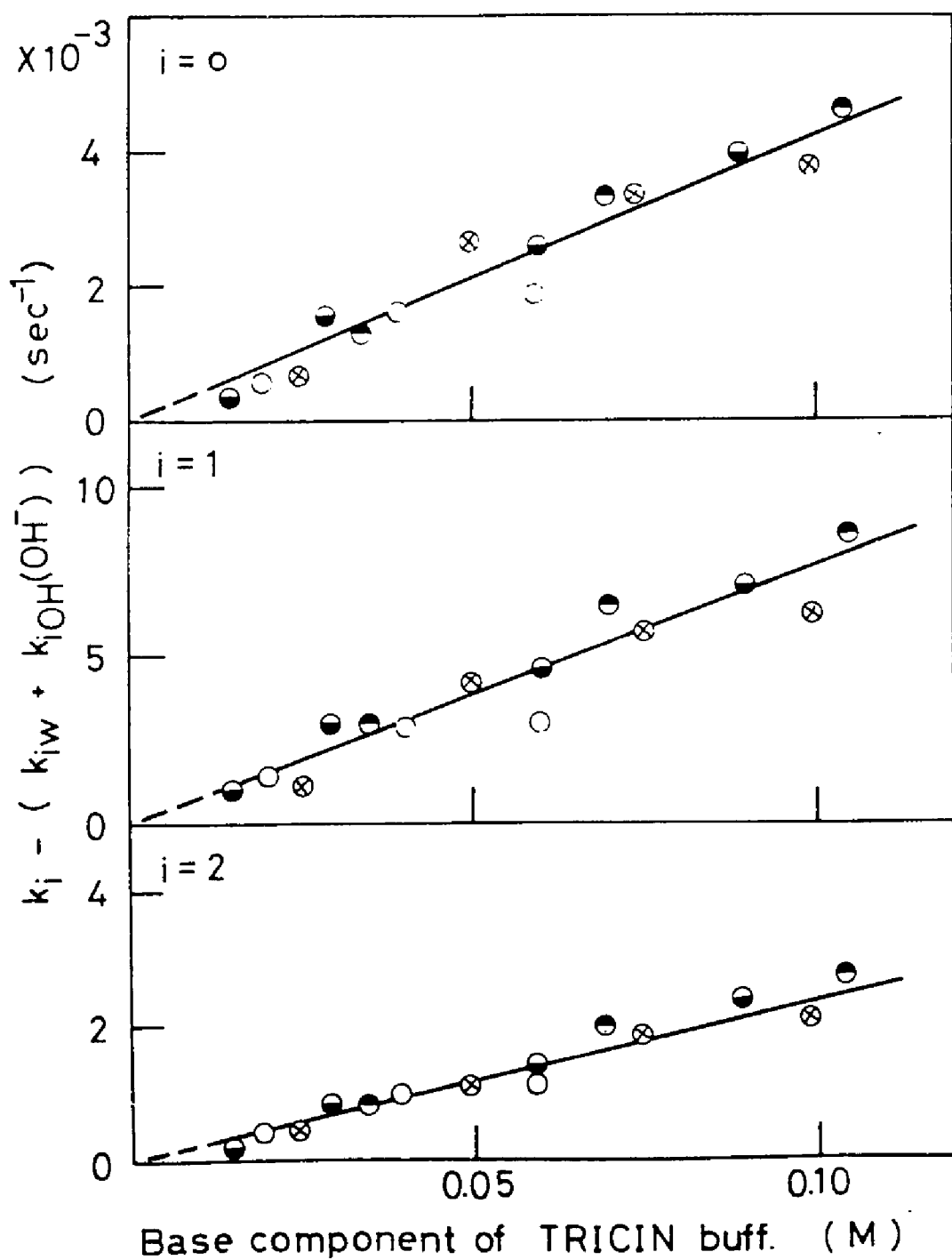


Fig.III-9 Plot of $k_i - (k_{i,w} + k_{i,\text{OH}}(\text{OH}^-))$ against the concentration of the base component of TRIS buffer. \circ : $N=1.0$, \bullet : $N=0.7$, \ominus : $N=1.5$.



Fgi.III-10 Plot of $k_i - (k_{i,w} + k_{i,OP(OH^-)})$ against the concentration of the base component of TRICIN buffer.

○: $N=1.5$, ⊗: $N=1.0$, ●: $N=0.7$, ◐: $N=0.4$.

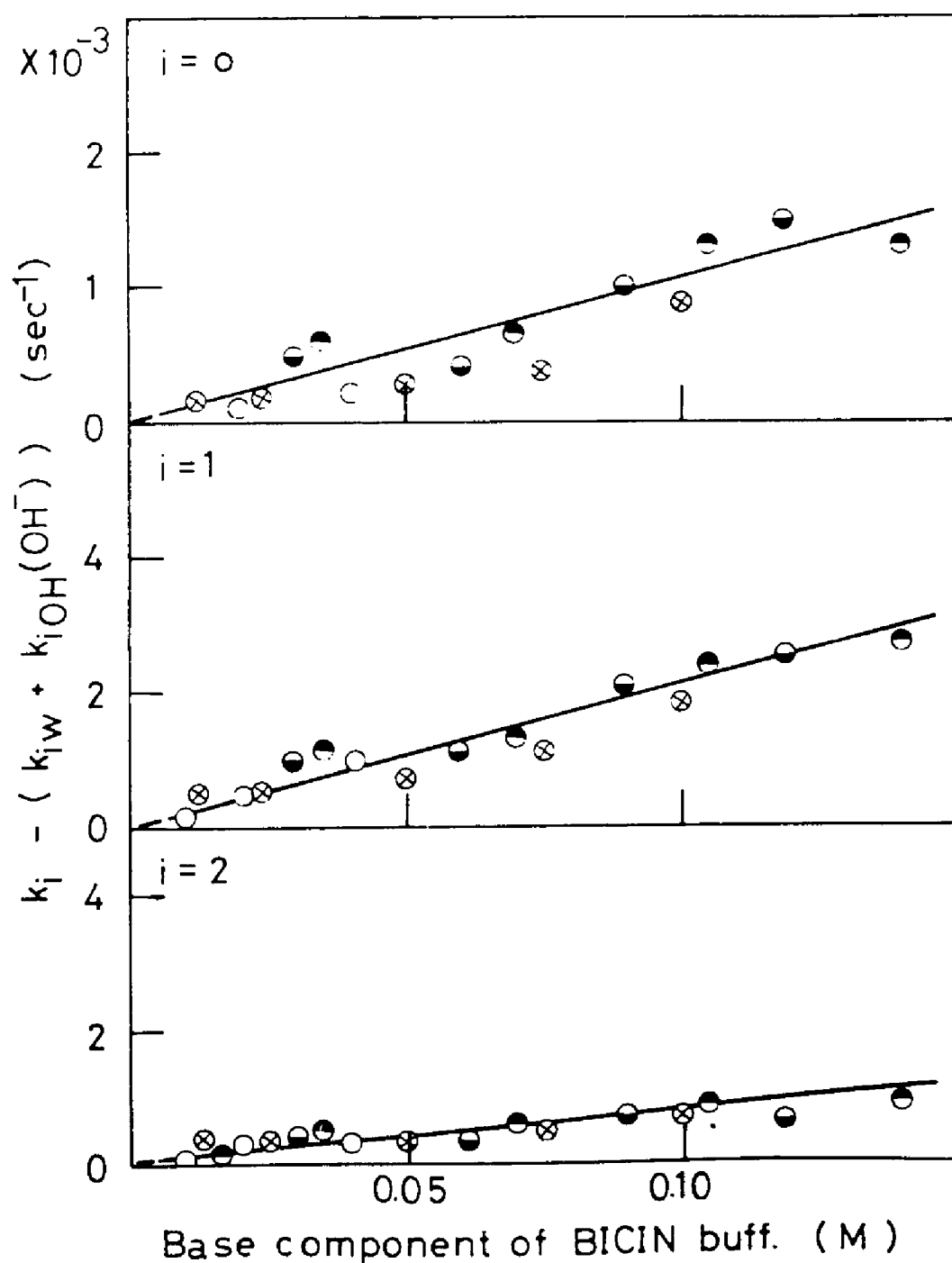


Fig.III-11 Plot of $k_i - (k_{i,w} + k_{i,OH(OH^-)})$ against the concentration of the base component of BICIN buffer
 ○: N=1.5, ⊗: N=1.0, ◐: N=0.7, ●: N=0.4.

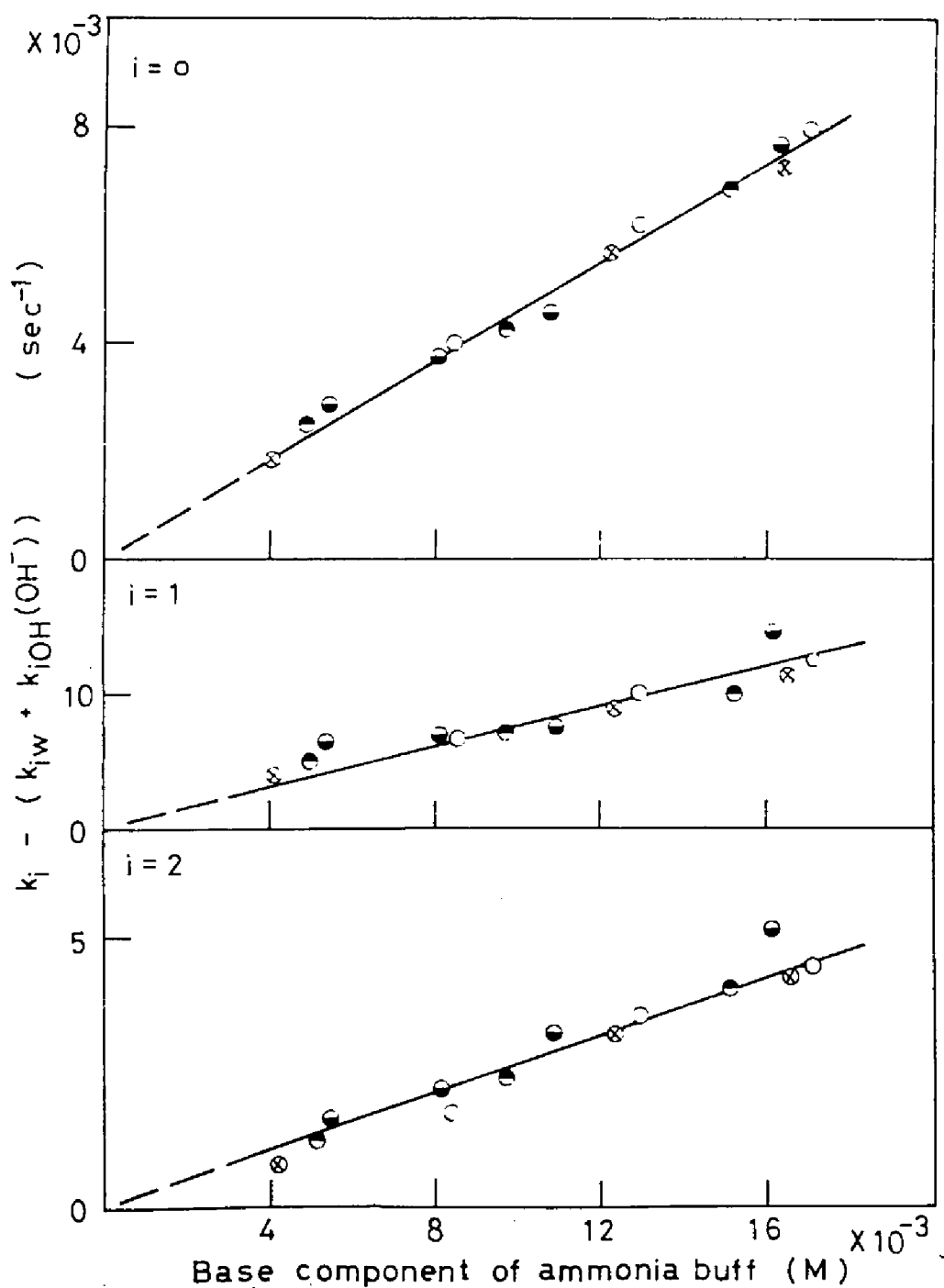


Fig.III-12 Plot of $k_i - (k_{i,w} + k_{i,OH}(OH^-))$ against the concentration of the base component of ammonia buffer.

○: $N=22.1$, ⊗: $N=11.1$ ●: $N=17.6$, ◐: $N=8.8$.

for H_2PO_4^- . It may be seen in Table III-5 that, except the rate coefficients for H_2PO_4^- , the rate coefficients, $k_{1,B}$, is always larger than the rate coefficients, $k_{2,B}$ where B is a base catalyst. In Fig. III-13, $\log k_{1,B}$ and $\log k_{2,B}$ are plotted against the pK_A value of the base catalyst, B. The rate coefficients for the water molecule, assumed to be a base catalyst, were calculated by means of $k_{i,w}/55.6\text{M}$ and plotted against $\text{pK}_A = -\log 55.6$.¹⁵⁾ The rate coefficients for H_2O , HPO_4^{2-} , NH_3 and OH^- are on a straight line with a slope of 0.42. The value of this slope seems reasonable in view of the value, 0.40 or 0.34, observed for the overall mutarotation rate constants of D-glucose.¹⁵⁾ The rate coefficients for the base components of TRIS, TRICIN, and BICIN are smaller than the theoretical values expected from the Brönsted plot and their pK' s and become smaller with an increase in their molecular size. These results should be attributed to the steric hindrance effect in the catalytic mutarotation reaction.^{31,33)} Smith⁵⁰⁾ and Los and Simpson^{19,20)} have pointed out that the catalytic effect caused by the sugar anion should generally be included. According to these authors, the rate coefficient for (OH^-) in Eq.(3-1) should be rewritten as:

$$k_{i,\text{OH}} = k'_{i,\text{OH}} + k_{i,\text{xyl}}(\text{xyl}) K_{\text{xyl}}/K_w$$

where k'_{OH} and $k_{i,\text{xyl}}$ are the true rate coefficients for OH^- and the xylosate anion respectively, (xyl) the concentration of D-xylose, K_{xyl} ($= 10^{-12.3}$)⁵¹⁾ the dissociation constant of the xylose \rightleftharpoons xylosate anion + H^+ , reaction, and K_w the ionic product of water. Applying the Brönsted rule (Fig. III-13) to D-xylose, one obtains $k_{1,\text{xyl}} \approx 30 \text{ sec}^{-1} \text{ M}^{-1}$. In the present experiments, (xyl) was 0.06M , so that $k_{1,\text{xyl}}(\text{xyl})K_{\text{xyl}}/K_w \approx 90 \text{ sec}^{-1} \text{ M}^{-1}$, which is approximately 8 % or less of $k_{1,\text{OH}}$. Accordingly, the effect by the xylosate anion may be considered to be of secondary significance in the present case. In reality, any appreciable experimental indication such as

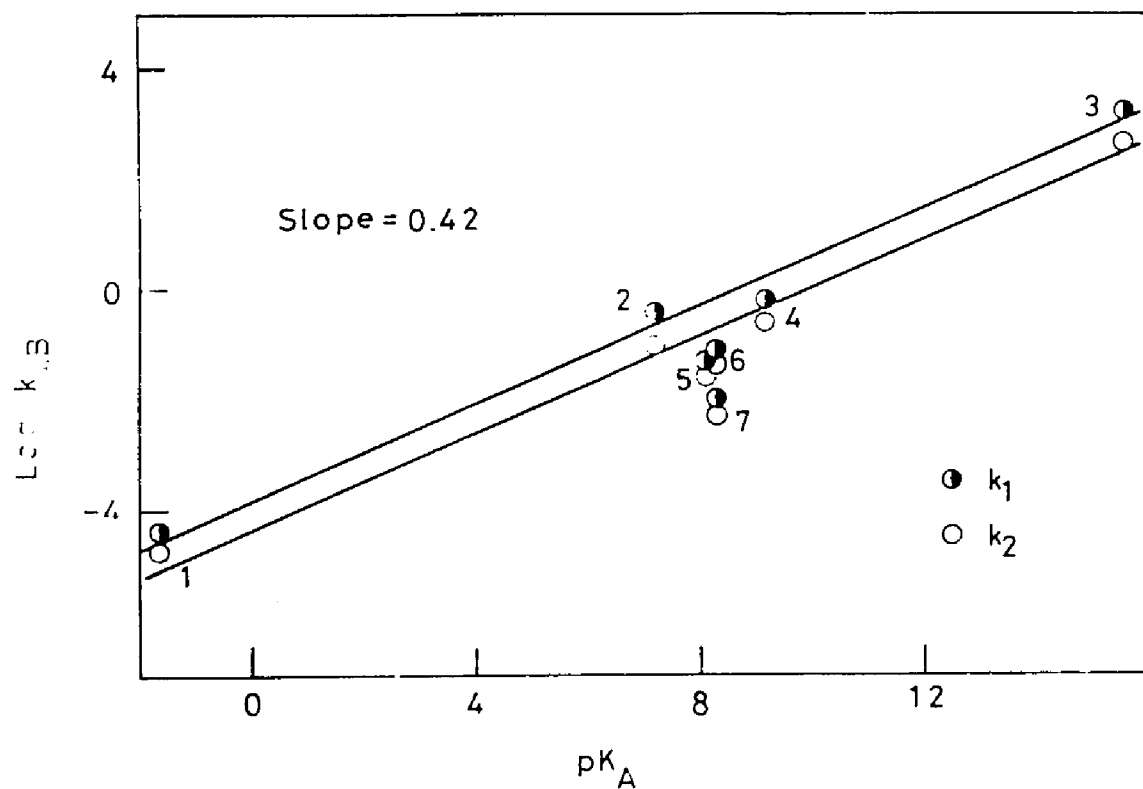


Fig.III-13 Brönsted plot for mutarotation of D-xylose. 1: H_2O , 2: HPO_4^{2-} , 3: OH^- , 4: NH_3 , 5: $(CH_2OH)_3-CNHCOP_2COO^-$, 6: $(CH_2OH)_3-CN H_2$, 7: $(C_2H_4OH)_2-NCH_2COO^-$.

TABLE III-5. THE OVERALL AND THE FORWARD RATE COEFFICIENTS
FOR THE MUTAROTATION OF D-XYLOSE AT 25°C

		Rate coefficient		
		i = 0	i = 1	i = 2
$k_{i,w}$	(sec ⁻¹)	1.4x10 ^{-3a)}	2.4x10 ⁻³	1.0x10 ⁻³
$k_{i,H_2PO_4^-}$	(sec ⁻¹ M ⁻¹)	0.2x10 ⁻²	0.3x10 ⁻²	1.7x10 ⁻²
$k_{i,HPO_4^{2-}}$	(")	2.1x10 ⁻¹	4.1x10 ⁻¹	1.0x10 ⁻¹
$k_{i,TRICIN}$	(")	4.2x10 ⁻²	7.7x10 ⁻²	2.4x10 ⁻²
$k_{i,TRIS}$	(")	0.7x10 ⁻¹	1.1x10 ⁻¹	0.4x10 ⁻¹
$k_{i,BICIN}$	(")	1.0x10 ⁻²	2.0x10 ⁻²	0.7x10 ⁻²
k_{i,NH_3}	(")	4.7x10 ⁻¹	7.6x10 ⁻¹	2.5x10 ⁻¹
$k_{i,OH}$	(")	0.7x10 ³	1.2x10 ³	0.4x10 ³

a) The value of $k_{o,w}$ obtained by polarimetry is 1.3x10⁻³sec⁻¹.

a nonlinear dependence of the polarographic current on (xyl) was not found of the significant contribution of xylosate anion to catalysis.

The ratio, R , of the concentration of γ -form to the concentration of the pyranose form is given in Table III-4. This value was found to change slightly with the change in the buffer concentration: for example, R for TRIS buffer changed 0.22 % to 0.40 % with the change in the buffer concentration from 0.20 to 0.02M, while R for phosphate buffer changed from 0.023 to 0.038 % with the change in the buffer concentration from 0.10 to 0.03M. Similar upward trend of R with a decrease in the buffer concentration has also been reported by previous authors.⁸⁾ Relatively large difference between R values obtained from the phosphate buffer and the amine buffers

should also be noted. These results suggest a further complicated structure of the intermediate form in the reaction mechanism of the mutarotation of monosaccharides.

Activation energy of the mutarotation reaction of α -D-xylose
The activation free energy, ΔG^\ddagger , the activation enthalpy, ΔH^\ddagger , and the activation entropy, ΔS^\ddagger , were calculated with the aid of the Eyring's equation

$$k = \kappa \frac{k_b T}{h} \exp(-\Delta G^\ddagger / RT) \quad (3-2)$$

where κ represents the transfer coefficient and h the Plank's constant, and k_b the Boltzmann's constant. The value of ΔE^\ddagger determined from the Arrhenius plot was revealed to be nearly equal to ΔH^\ddagger value. The results are shown in Table III-6 and III-7. From Table III-6, it can be seen that the difference of the catalytic effect between phosphate anion and water molecule is mainly due to the difference in the entropy term between these two species. Table III-7 shows that the difference of the enthalpy ΔH , between pyranose form and γ -form is about 10 Kcal. mol⁻¹. The difference in entropy term between forward (α - or β -pyranose form to γ -form) reaction and the backward (γ -form to α - or β -pyranose form) reaction is about 20 e.u. This permit to suppose that the entropy of " γ -form" is larger than those of α - and β -pyranose. These results suggest a reaction mechanism containing several acyclic intermediates.

SUMMARY

The polarographic behavior of α -D-xylose in several buffer solutions was investigated. The individual and the overall rate constants of the mutarotation of α -D-xylose in various buffer solutions were determined. The overall and the forward rate constants, were analyzed as a linear function of the buffer concentration.

TABLE III-6. ACTIVATION ENERGY FOR THE OVERALL MUTAROTATION
REACTION OF D-XYLOSE

Catalyst	ΔG^{\ddagger} (Kcal/mol)	ΔH^{\ddagger} (Kcal/mol)	ΔS^{\ddagger} (cal/mol.K)
H ₂ O	23.7	16.2	-25
HPO ₄ ²⁻	18.4	16.9	-5.1

TABLE III-7. ACTIVATION ENERGY FOR THE MUTAROAATION REACTION
($\alpha = \gamma = \beta$) OF D-XYLOSE CATALYZED BY HPO₄²⁻.

Reaction	ΔG^{\ddagger} (Kcal/mol)	ΔH^{\ddagger} (Kcal/mol)	ΔS^{\ddagger} (cal/mol.K)
$\alpha \rightarrow \gamma$	18.0	16.4	-5.4
$\beta \rightarrow \gamma$	18.8	16.7	-7.1
$\gamma \rightarrow \alpha$	14.0	6.2	-26
$\gamma \rightarrow \beta$	14.2	7.1	-24

It was revealed that the catalytic effects of the buffer salts are mainly due to their basic components for all the buffer salts investigated. The Brönsted plots of k_1 and k_2 for all the basic components gave straight lines with the same slope of 0.42. The rate constants for several amines deviated from the slope; this deviation was ascribed to the steric hindrance caused by the large molecular sizes.

The activation free energies, enthalpies, and entropies of the mutarotation of α -D-xylose in a neutral phosphate buffer solution were determined.

CHAPTER IV. MECHANISM OF ACID-BASE CATALYZED MUTAROTATION OF MONOSACCHARIDES

As pointed out in Chapters II and III, the relative amount of intermediate γ -form depends on both kinds and concentrations of the buffer salts. The equilibrium ratio, R , of the intermediate γ -form to α - and β -pyranose form in ammonia buffer is about 10 times larger in comparison with that in phosphate buffer (Table IV-1). The value of R also depends on the concentration of buffer salts. Typical examples are shown in Table IV-1. Recently, Fonds and Los⁵²⁾ reinvestigated the electro-reduction of α -D-glucose by use of pulse polarography. They ascribed this dependence of the relative amount of γ -form on the buffer concentrations to the choice of an erroneous diffusion coefficient which was employed for the calculation of the backward reaction rate, k_{-1} and k_{-2} in Eq.(2-2). They selected a set of diffusion coefficient of D-glucose so that R becomes constant. According to their results, the diffusion coefficient of D-glucose in 0.2 M phosphate buffer solution becomes a half of the diffusion coefficient in pure water. This decrease of the diffusion coefficient was ascribed to the association of γ -form with phosphate ions. Provided that this is also valid for other kinds of buffer salts, the diffusion coefficient of γ -form in ammonia buffer should be about a tenth of that in phosphate buffer. This is unlikely. Thus we must seek for another reason.

In this chapter, the mechanism of the acid-base catalyzed mutarotation reaction of monosaccharides is discussed on the basis of the experimental results obtained in this study.

FORWARD REACTION

As described in Chapter III, the forward or ring opening reaction (α - or β -pyranose to γ -form) are catalyzed mainly by the basic components of buffer salt. The rate constants, k_1 and k_2 ,

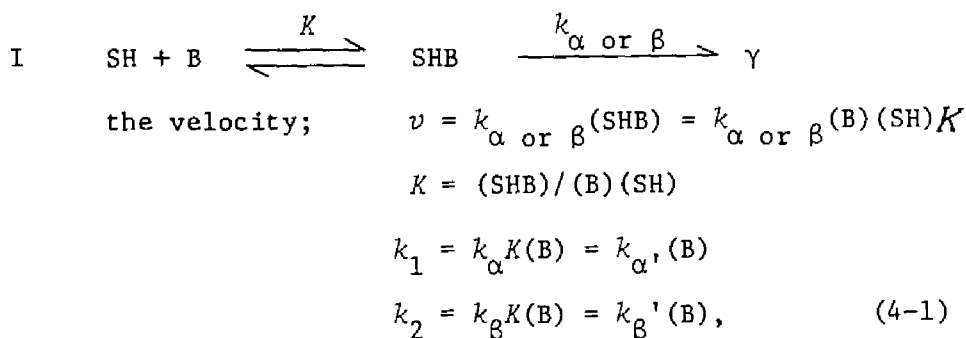
TABLE IV-1. RATIO, R , OF THE CONCENTRATION OF INTERMEDIATE γ -FORM TO THE CONCENTRATION OF PYRANOSE FORM

D-glucose		D-xylose			
Phosphate buff. of pH 6.9 (M)	$R(\%)$	Phosphate buff. of pH 6.6 (M)	$R(\%)$	Ammonia buff. of pH 8.6 (M)	$R(\%)$
0.07	0.0037 ^{**} (0.0028 [*])	0.03	0.038 (0.026 [*])	0.05	0.36 (0.22 [*])
0.11	0.0032 ^{**} (0.0025 [*])	0.05	0.032 (0.022 [*])	0.10	0.32 (0.19 [*])
0.15	0.0027 ^{**} (0.0024 [*])	0.07	0.027 (0.020 [*])	0.15	0.29 (0.17 [*])
0.19	0.0025 ^{**} (0.0023 [*])	0.10	0.025 (0.019 [*])	0.20	0.26 (0.16 [*])
0.25	0.0027 ^{**} (0.0022 [*])			0.25	0.24 (0.15 [*])

* Calculated by Eq. (4-7).

** The data from ref. 52)

are linear function of the concentration of the base components. These results are in good agreement with the accepted concept of the general acid-base catalysis. Accordingly, a possible mechanism will be⁵³⁾

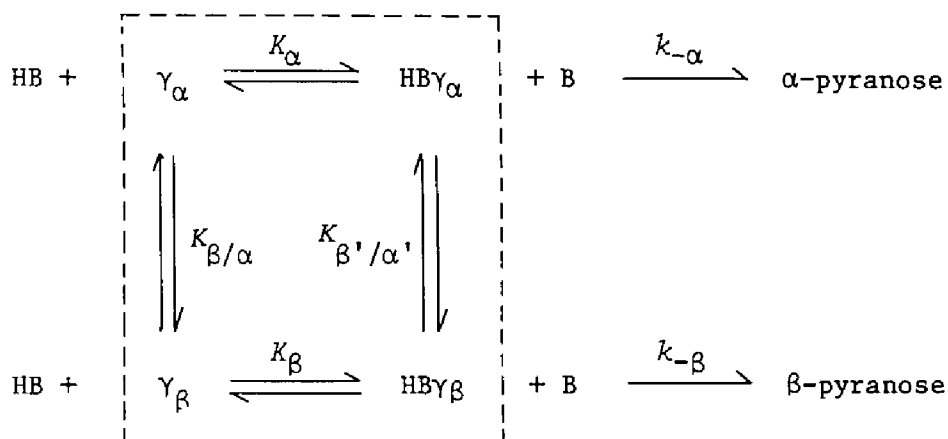


where SH represents α -pyranose form or β -pyranose form, B the base and SHB the intermediate complex, in which a hydrogen bonding between SH and B might be assumed. The fact that a slight drop of pH value is observed by the dissolution of sugar into the buffer solution is in favor of the supposed mechanism.

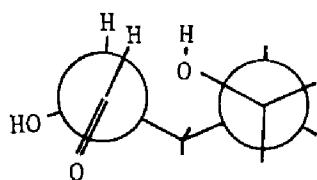
BACKWARD REACTION

The backward or ring closing rate constants, k_{-1} and k_{-2} , are not a linear function of the concentration of buffer salts. The amount of the intermediate γ -form calculated by use of these rate constants, depends on both kinds and concentrations of buffer salts (see *e.g.* Table IV-1). The experimental results of the activation energy for the mutarotation reaction also suggest the possibility to suppose a reaction mechanism involving several intermediate forms. A possible reaction mechanism would be

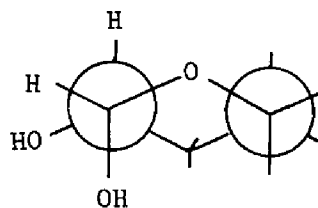
II



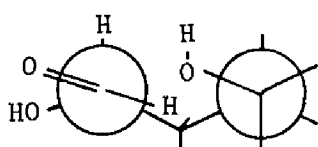
(γ -form)



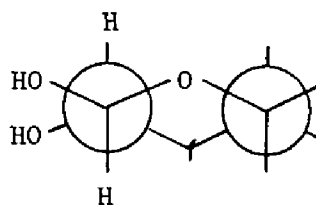
γ_{α}



α -pyr.



γ_{β}



β -pyr.

where γ_{α} and γ_{β} are two of the intermediate forms similar to α -pyranose and β -pyranose in conformation, but have acyclic structures respectively and $\text{HB}\gamma_{\alpha}$ and $\text{HB}\gamma_{\beta}$ are the forms in which HB is hydrogen-bonded with C-1 oxygen of γ_{α} and γ_{β} , respectively. Here, it is assumed that four intermediate forms, γ_{α} , γ_{β} , $\text{HB}\gamma_{\alpha}$, and $\text{HB}\gamma_{\beta}$, lie in

a partial equilibrium with each other; the equilibrium constants are defined by $K_{\beta/\alpha} = (\gamma_{\beta})/(\gamma_{\alpha})$, $K_{\beta'/\alpha'} = (\text{HB}\gamma_{\alpha})/(\text{HB}\gamma_{\beta})$, $K_{\alpha} = (\text{HB}\gamma_{\alpha})/(\text{HB})(\gamma_{\alpha})$, and $K_{\beta} = (\text{HB}\gamma_{\beta})/(\text{HB})(\gamma_{\beta})$.

The expression for the velocity of this reaction mechanism is:

$$v_{-\alpha} = k_{-\alpha}(\text{B})(\text{HB}\gamma_{\alpha}) = k_{-\alpha}K_{\alpha}(\text{B})(\text{HB})(\gamma_{\alpha})$$

$$v_{-1} = \frac{k_{-\alpha}K_{\alpha}(\text{HB})(\text{B})}{(1 + K_{\beta/\alpha})(1 + K_{\alpha}(\text{HB}))} \quad (4-2)$$

$$v_{-\beta} = k_{-\beta}(\text{B})(\text{HB}\gamma_{\beta}) = k_{-\beta}K_{\beta}(\text{B})(\text{HB})(\gamma_{\beta})$$

$$k_{-2} = \frac{k_{-\beta}K_{\beta}(\text{HB})(\text{B})}{(1 + 1/K_{\beta/\alpha})(1 + K_{\beta}(\text{HB}))} \quad (4-3)$$

where $K_{\beta'/\alpha'} \approx K_{\beta/\alpha}$ (i.e. $K_{\alpha} \approx K_{\beta}$) is assumed. By using the total concentration of the buffer components, defined by $C = (\text{HB}) + (\text{B})$, and the ratio, defined by $N = (\text{HB})/(\text{B})$, Eq.(4-2) can be rewritten as:

$$\frac{C}{k_{-1}} = (1 + N) \frac{1 + K_{\beta/\alpha}}{k_{-\alpha}} + \frac{(1 + N)^2}{N} \frac{1 + K_{\beta/\alpha}}{k_{-\alpha}} \frac{1}{K_{\alpha}} \frac{1}{C} \quad (4-4)$$

When the value of C/k_{-1} is plotted against $1/C$ at a given N value, a straight line will be obtained; the slope of this line is $(1+N)^2/N \cdot (1+K_{\beta/\alpha})/k_{-\alpha} \cdot 1/K_{\alpha}$, and the intercept on the C/k_{-1} axis is $(1+N)(1+K_{\beta/\alpha})/k_{-\alpha}$. If we assume that the value of $K_{\beta/\alpha}$ is about the same with the value of the equilibrium ratio of β -pyranose to α -pyranose, we can calculate K_{α} and $k_{-\alpha}$ from the values of this slope and intercept. A similar equation to Eq.(4-4) can also be obtained from Eq.(4-3) and we can calculate K_{β} and $k_{-\beta}$ likewise. Fig.IV-1 shows the plot of C/k_{-1} against $1/C$ for D-xylose in ammonia and phosphate buffer solutions. The values of $k_{-\alpha}$, $k_{-\beta}$, and K_{α} and K_{β} obtained from these plots are shown in Table.IV-2 together with k_{α} and k_{β} , which have been obtained in Chapter III (Table III-5). Similar plot for D-glucose

in a phosphate buffer solution is shown in Fig.IV-2, for which data are taken from Fonds and Los's report.⁵²⁾ The values of the rate constants and equilibrium constants obtained by the same way are also shown in Table IV-2. The K_α values are in the same order with the K_β values in each case. Thus, the initial assumption that $K_\alpha \approx K_\beta$ is approximately satisfied. The forward rate constants, k_α , and k_β , for D-xylose are larger than those for D-glucose, while the backward rate constants, $k_{-\alpha}$ and $k_{-\beta}$, obtained for D-xylose are smaller than those for D-glucose. The result that K_α and K_β in phosphate buffer are larger than K_α and K_β in ammonia buffer, respectively, for D-xylose is understandable because the acid component of phosphate buffer has the stronger acidity than that of ammonia buffer.

The equilibrium ratio, R , of the total concentration (γ) of the intermediate forms (defined by $(\gamma) = (\gamma_\alpha) + (\gamma_\beta) + (\text{HB}\gamma_\alpha) + (\text{HB}\gamma_\beta)$) to the concentration of pyranose form can be calculated according to the following equation:

$$R = \frac{1}{\frac{k_{-\alpha}}{k_\alpha (1+K_{\beta/\alpha}) (1+1/K_\alpha (\text{HB}))} + \frac{k_{-\beta}}{k_\beta (1+1/K_{\beta/\alpha}) (1+1/K_\beta (\text{HB}))}} \quad (4-5)$$

The values of R calculated by use of Eq.(4-5) with k 's and K 's as given in Table IV-2, are shown in Table IV-1 for the comparison. The downward trend of R with increasing buffer concentration is reasonably explained.

If the proposed mechanism is valid, the equilibrium constant, $K = (\beta\text{-pyranose})/(\alpha\text{-pyranose})$ should be given by

$$K = \frac{k_\alpha}{k_\beta} \cdot \frac{k_{-\beta}}{k_{-\alpha}} \cdot \frac{(1+K_{\beta/\alpha})}{(1+1/K_{\beta/\alpha})} \cdot \frac{K_\beta}{K_\alpha} \cdot \frac{(1+K_\alpha (\text{HB}))}{(1+K_\beta (\text{HB}))} \quad (4-6)$$

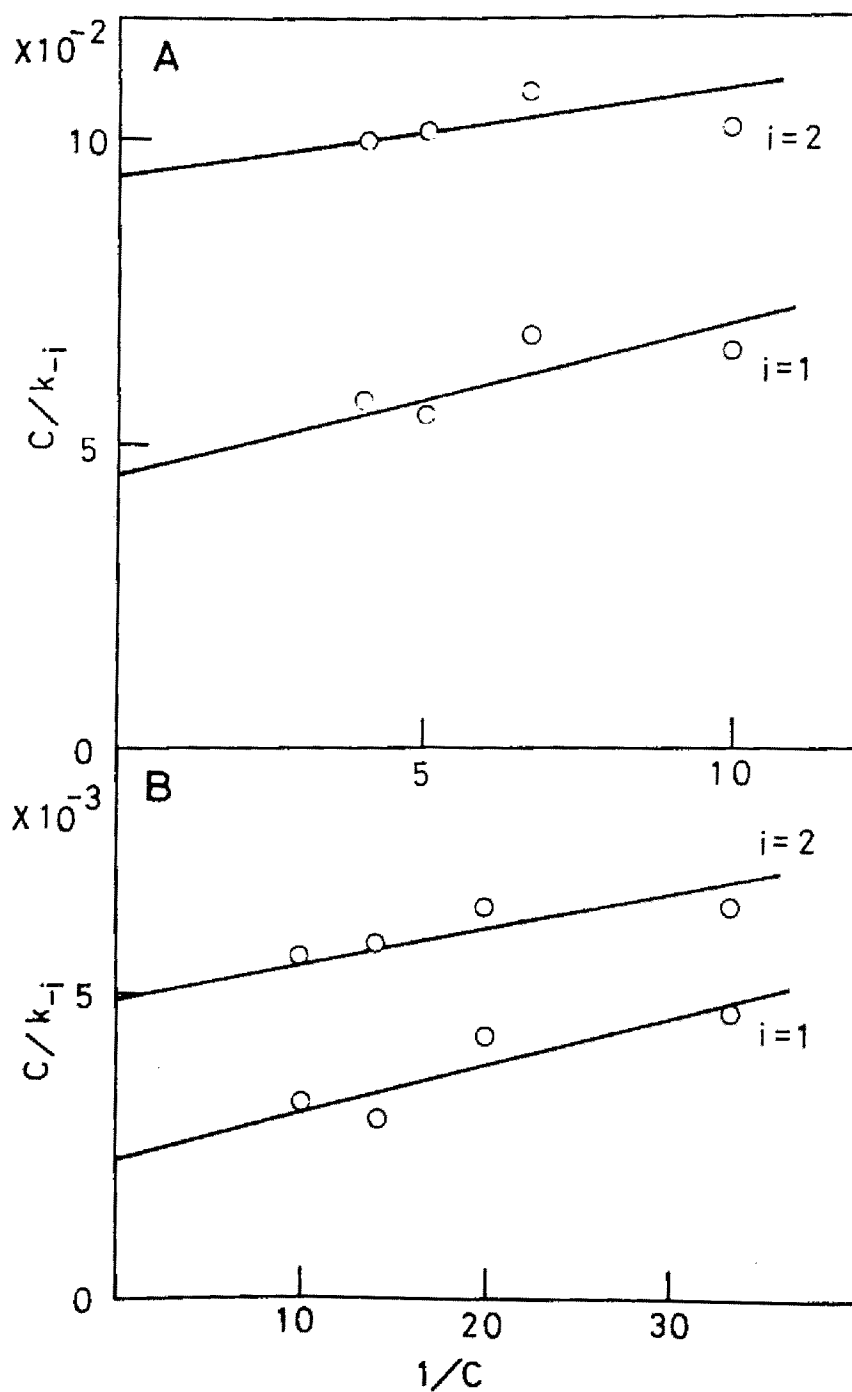


Fig.IV-1 Plot of C/k_{-i} against $1/C$ for D-xylose. A: Ammonia buffer, B: Phosphate buffer.

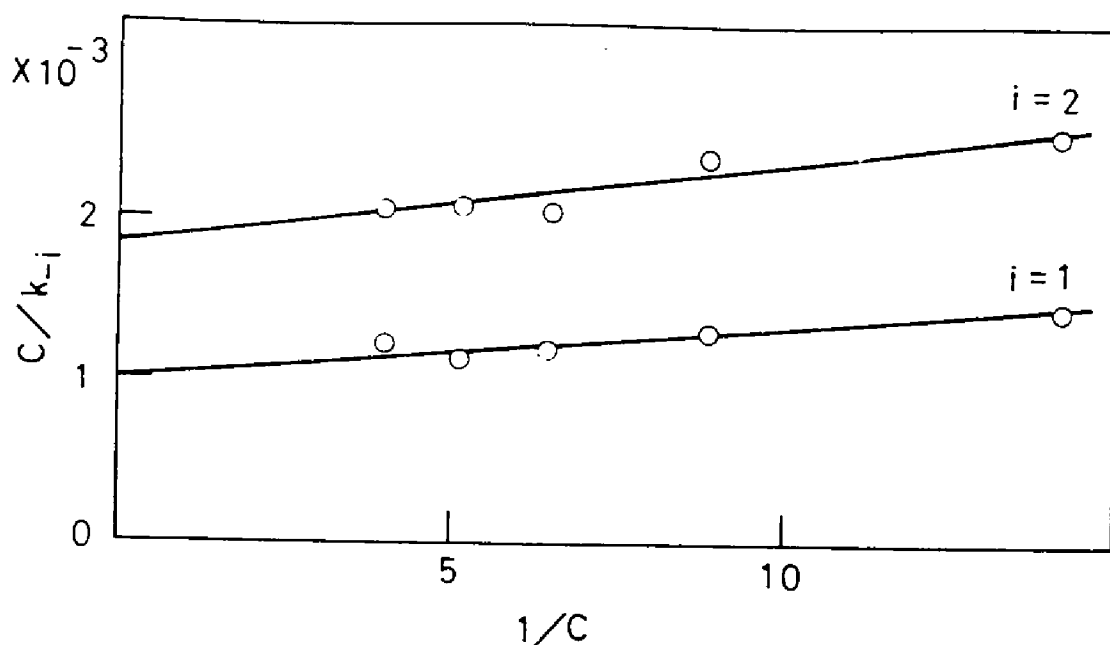


Fig.IV-2 Plot of C/k_{-i} against $1/C$ for D-glucose in phosphate buffer.

The value of K were calculated to be 2.0 ± 0.5 for D-xylose in the range of 0.03 to 0.10 M phosphate buffers (pH 6.6) and 0.05 to 0.25 M ammonia buffers (pH 8.6) and 1.6 ± 0.2 for D-glucose in the range of 0.07 to 0.25 M phosphate buffers (pH 6.9). Namely the values of K are practically independent of pH and buffer concentrations. These results are reasonably in agreement with the experimental results.

In conclusion, it may be stated that the experimental results for the backward reaction obtained in the present studies can reasonably explained by the mechanism (II) mentioned above. However, at present it is difficult to harmonize stoichiometrically the backward reaction mechanism (II) with the forward reaction mechanism (I). Further studies are wanted.

TABLE IV-2. KINETIC PARAMETERS OF MUTAROTATION

	$k_{\alpha'}$	$k_{\beta'}$	$k_{-\alpha}$	$k_{-\beta}$	K_{α}	K_{β}
D-xyl. in phosphate buffer	0.41	0.10	310	270	58	147
D-xyl. in ammonia buffer	0.76	0.25	79	64	21	80
D-glu. in phosphate buffer	0.071	0.025	518	472	116	154

SUMMARY

The mechanism of the acid-base catalyzed mutarotation of monosaccharides was discussed on the basis of the experimental results obtained in Chapter II and Chapter III. A probable mechanism is proposed, in which four reaction intermediates are assumed.

CHAPTER V. POLAROGRAPHIC STUDY OF MONOSACCHARIDES IN UNBUFFERED SOLUTION AND ITS APPLICATION TO THE DETERMINATION OF MUTAROTATION RATE CONSTANTS

Little attention has been paid to the polarographic behavior of monosaccharides in unbuffered solution. As noted briefly in Chapter III, the author has found that in unbuffered solutions the polarographic wave of monosaccharide gave a large limiting current, which seemed to be an autocatalytic current. This chapter is concerned with the elucidation of the characteristic behavior of the polarographic wave of monosaccharide in unbuffered media. An application of the wave to the determination of the mutarotation rate constants of monosaccharide is also described in this chapter.

EXPERIMENTAL

Materials Reagent-grade D-glucose, D-galactose, and D-xylose were obtained commercially. Pure α -D-glucose ($[\alpha]_D^{25} = +112$) was a gift from Tokai Togyo Co.Ltd. Pure α -D-galactose and α -D-xylose were prepared by recrystallization from hot ethanol and water respectively⁴⁶⁾ (α -D-galactose: $[\alpha]_D^{25} = +150$, α -D-xylose: $[\alpha]_D^{25} +94$). Reagent grade potassium chloride and tetraethylammonium-chloride (TEAC) used as supporting electrolytes were obtained commercially. The latter was used after recrystallization from methanol.

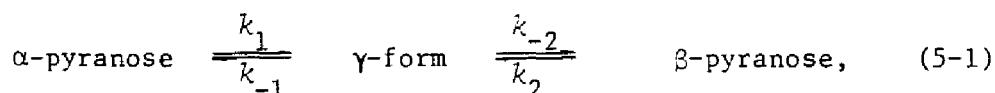
Apparatus Polarograms were recorded with a Yanagimoto PE21-TB2S polarograph connected with a Yokokawa 3077 X-Y recorder. Polarimetry was carried out with a Yanagimoto polarimeter OR-20.

The measurements were made with an H-type cell. A saturated calomel electrode (SCE) was used as reference electrode. In order to minimize an accumulation of hydroxyl ion in the close neighbour-

hood of the electrode, which could be caused by unsufficient stirring of the neighbouring solution after the detachment of the individual mercury drops, a pencil-sharpened capillary (tip outside-diameter 1 mm) was used. The capillary characteristics of the dropping mercury electrode measured in 0.1M potassium chloride (open circuit) at a mercury reservoir height of 52.5 cm were $m = 1.306 \text{ mg sec}^{-1}$ and $\tau = 5.65 \text{ sec}$. All experiments were carried out at $25^\circ \pm 0.05^\circ \text{C}$. The procedure for the measurement of the polarographic current is the same as described in Chapter II.

THEORY

Limiting current As described in Chapter II, the strictly rate-controlled limiting current i_1 , for the reaction scheme (5-1):



is expressed by

$$\bar{i}_1 = nF\bar{q}\bar{\mu}(k_1C_\alpha + k_2C_\beta) \quad (5-2)$$

$$\bar{\mu} = (D_s/(k_{-1} + k_{-2}))^{1/2} \quad (5-3)$$

where D_s is the diffusion coefficient of monosaccharide, C_α and C_β the bulk concentration of α - and β -pyranose, respectively. The rate constants k_i ($i = 1, 2, -1$, and -2) of Eq.(5-1) depend on the concentrations (activities) of acid and base catalysts.

In unbuffered aqueous solutions, they are generally expressed by^{15,16)}

$$k_i = k_{i,w} + k_{i,H}(H^+) + k_{i,OH}(OH^-), \quad (5-4)$$

($i = 1, 2, -1$, and -2)

where $k_{i,w}$ represents the catalytic constant of water, $k_{i,H}$ and $k_{i,OH}$ the catalytic coefficients of the catalysts indicated by subscripts, and the symbols in brackets the concentrations (activities) of the catalysts, H^+ and OH^- being hydrogen ion and hydroxyl ion, respectively.

In the reaction layer the solution is basic because of the production of hydroxyl ions by the electro-reduction of monosaccharide at the electrode surface. For example, a rough estimation shows pH is 9.8 at the electrode when 0.006M D-xylose solution is reduced at d.m.e. in unbuffered solution. The catalytic effect of hydrogen ion may be neglected in such a basic solution. The first term on the right-hand side of Eq.(5-4), $k_{i,w}$ may also be negligible in comparison with the third term. Accordingly, the rate constants, k_i ($i=1,2,-1$, and -2), in the reaction layer are eventually expressed, in the first approximation, by

$$k_i = k_{i,OH}(OH^-)^0, \quad (5-5)$$

where $(OH^-)^0$ represents the mean concentration of hydroxyl ions at the electrode surface, which may be assumed to be approximately constant throughout the reaction layer because of the thinness of the layer.

Since hydroxyl ions diffuse into the solution and the concentration of hydroxyl ion is practically zero in the bulk solution, the limiting current of monosaccharide is related to the concentration of hydroxyl ions at the electrode surface, with the aid of the Ilkovic equation:⁴⁸⁾ i.e.

$$\bar{i}_1 = \bar{\kappa}_{OH}(OH^-)^0, \quad (5-6)$$

where $\bar{\kappa}_{OH}$ is the Ilkovic constant for hydroxyl ion.

Substitution of Eqs.(5-5) and (5-6) into Eq.(5-2) gives:

$$\bar{i}_1 = 1.322 \bar{\kappa}_s (D_s/D_{OH})^{1/2} \tau (k_{-1,OH} + k_{-2,OH})^{-1} (k_{1,OH} C_\alpha + k_{2,OH} C_\beta)^2, \quad (5-7)$$

where $\bar{\kappa}_s$ is Ilkovic constant for the hypothetical diffusion current of monosaccharide with $n=2$ ($\bar{\kappa}_s = 2(D_s/D_{OH})^{1/2} \bar{\kappa}_{OH}$), D_{OH} the diffusion coefficient of hydroxyl ion. For the solution of the equilibrated mixture of monosaccharide, Eq.(5-7) is reduced to

$$\begin{aligned} \bar{i}_1 = 1.322 \bar{\kappa}_s (D_s/D_{OH})^{1/2} \tau (k_{1,OH} + k_{2,OH} K)^2 (C_{\alpha,eq} + C_{\beta,eq})^2 \times \\ \times (k_{-1,OH} + k_{-2,OH})^{-1} (1+K)^{-2}, \end{aligned} \quad (5-7')$$

where $C_{\alpha,eq}$ and $C_{\beta,eq}$ are the equilibrium concentrations of α - and β -pyranose, respectively; and K is the equilibrium constant defined by $K = C_{\beta,eq}/C_{\alpha,eq}$. The sum $C_{\alpha,eq} + C_{\beta,eq}$ is equal to the total concentration of monosaccharide because the concentration of the intermediate γ -form is negligibly small.

Electrode reaction, conceptionally, of the same mechanism as that described above was previously proposed for the electro-reduction of formaldehyde in unbuffered solution by Brdicka⁴⁸⁾ and a mathematically rigorous solution of the process has been given by Koutecky.⁵⁵⁾ According to Koutecky's result, the numerical coefficient of Eq.(5-7') 1.322, should be replaced by 0.966.

Current Potential Curve Since the electroactive intermediate γ -form is formed from α - and β -pyranose, the current at any point of the kinetic wave of monosaccharide can be given by

$$\bar{i} = nF\bar{q}\bar{\mu} (k_1 C_\alpha^0 + k_2 C_\beta^0 - (k_{-1} + k_{-2}) C_\gamma^0), \quad (5-8)$$

where C_α^0 , C_β^0 and C_γ^0 represent the mean concentration of α -, β - and γ -form in the reaction layer, respectively. Provided that the limiting current has a strictly kinetic character as given by Eq.(5-2),

there is no significant depletion of α - and β -pyranose in the reaction layer. Then substitution of Eq.(5-2) in Eq.(5-8) gives:

$$\bar{i} = \bar{i}_1 - nF\bar{q}\bar{\mu}(k_{-1} + k_{-2})C_Y^0 \quad (5-8')$$

Based on the theory of slow discharge, the current intensity can be given by

$$\bar{i} = nF\bar{q}k_{el} \exp((- \alpha_a n_a F/RT)(E-E_o))C_Y^0, \quad (5-9)$$

where k_{el} represents the standard rate constant at the standard potential E_o , $\alpha_a n_a$ the product of the transfer coefficient and the number of electrons in the discharge process.

In unbuffered solutions the current intensity can also be related to the concentration of hydroxyl ion at the electrode surface by Eq.(5-6')

$$\bar{i} = \bar{\kappa}_{OH}(OH^-)^0 \quad (5-6')$$

Substituting the appropriate values from Eqs. (5-5), (5-6') and (5-9) into Eq(5-8'), we obtain after rearrangement

$$E = E_o + \frac{RT}{\alpha_a n_a F} \ln \left[\frac{k_{el} (\bar{\kappa}_{OH})^{1/2}}{(D_s (k_{-1,OH} + k_{-2,OH}))^{1/2}} \right] + \frac{RT}{\alpha_a n_a F} \ln \left[\frac{\bar{i}_1 - \bar{i}}{\bar{i}^{3/2}} \right] \quad (5-10)$$

here it is assumed that Eq.(5-5) is valid in this case.

RESULTS AND DISCUSSION

Experiments were carried out for several monosaccharides (D-glucose, D-galactose and D-xylose) in unbuffered solution of 0.1 M potassium chloride.

Curve A in Fig.V-1 shows an example of the polarographic wave

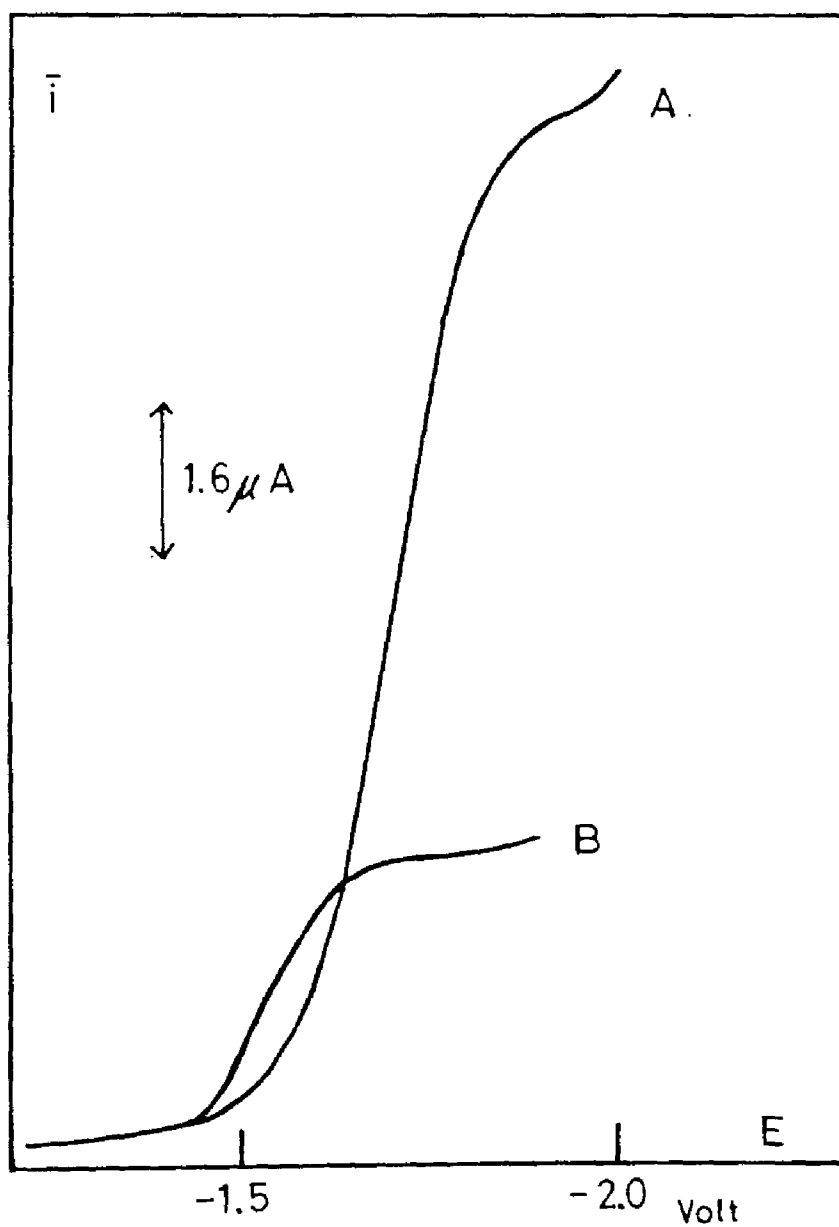


Fig.V-1 Polarograms of 0.06 M D-xylose in 0.1 M potassium chloride (A), and in 0.2 M ammonia buffer of pH 8.60 (B) at 25°C.

of D-xylose in unbuffered neutral solution. This wave is very large in comparison with the wave of D-xylose of the same concentration (Curve B in Fig.V-1) in the buffered solution of 0.2 M ammonia at pH 8.59. Similar large waves were observed for all other monosaccharides studied. The value of \bar{i}_1/\bar{i}_d is shown in Table V-1, \bar{i}_d being the hypothetical diffusion current for monosaccharide. The limiting current \bar{i}_1 is still small enough to be considered as strictly kinetic-controlled in the concentration ranges studied. Accordingly, the use of Eq.(5-2) in deriving the theoretical equations is justified.

It is expected from Eq.(5-7') that \bar{i}_1 is approximately proportional to $m^{2/3}\tau^{7/6}$. Since m is proportional to the height of the mercury reservoir, h and τ is inversely proportional to h , \bar{i}_1 is eventually proportional to $h^{-1/2}$. Experimental results (Table V-1) show that \bar{i}_1 decreases with the increase in the height of the mercury reservoir and is approximately proportional to $h^{-1/2}$.

TABLE V-1. DEPENDENCE OF THE LIMITING CURRENT, \bar{i}_1 , ON THE HEIGHT OF MERCURY RESERVOIR, h AT 25°C

h (cm)	D-glu. (60mm)		D-gal. (14mm)		D-xyl. (10mm)	
	\bar{i}_1 (cm)	$\bar{i}_1(h^{1/2})$	\bar{i}_1 (cm)	$\bar{i}_1(h^{1/2})$	\bar{i}_1 (cm)	$\bar{i}_1(h^{1/2})$
42.5	5.16	34	4.26	28	4.51	29
52.5	4.80	35	4.16	30	4.25	31
62.5	4.45	35	3.87	31	4.10	32
<i>c.f</i>						
\bar{i}_1/\bar{i}_d^*	2.1×10^{-2}		3.0×10^{-3}		1.3×10^{-2}	

* At $h=52.5$ cm, Current sensitivity: 0.2 μ A/cm.

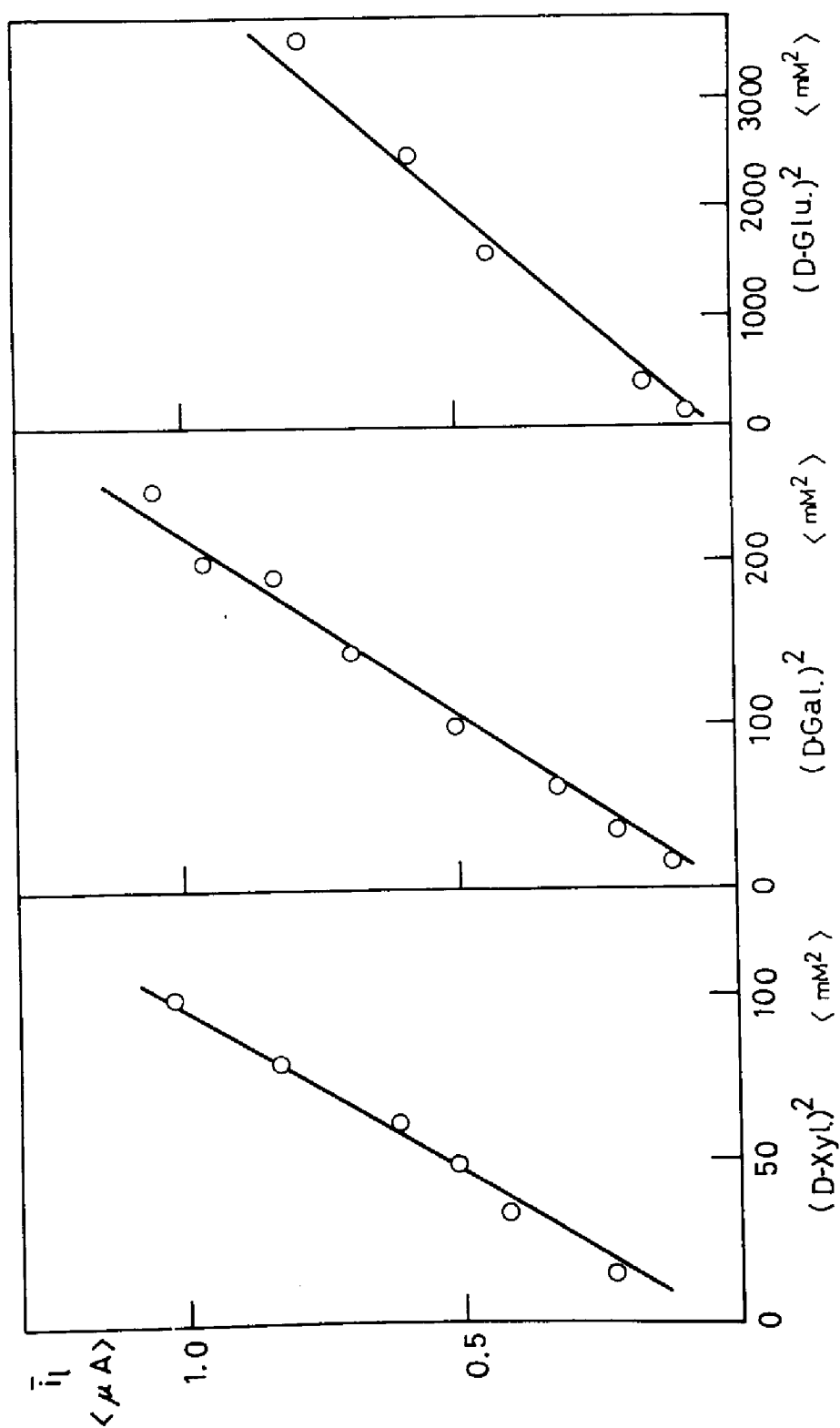


Fig.V-2 Relationship between the limiting current and the square of the concentration of monosaccharides in 0.1M potassium chloride.

Fig. V-2 shows the plot of \bar{i}_1 against the square of the monosaccharide concentration, $(C_\alpha + C_\beta)^2$. In accordance with the theoretical consideration, all the data in Fig.V-2 are distributed along a straight line; but at higher concentrations of monosaccharides, the data deviate downward from the line. Similar deviation of the data from linearity has also been observed for the limiting current of formaldehyde in unbuffered solution by Brdicka,⁴⁸⁾ who attributed the deviation to the streaming of the solution in the neighbourhood of the electrode.

The concentration of the hydroxyl ions in the reaction layer, $(OH^-)^0$, can be calculated with the aid of the Ilkovic equation (5-6). Even at the lowest concentration studied (0.04 M D-xylose), \bar{i}_1 reached 0.23 μA . Thus, $(OH^-)^0$ may be estimated as 3.6×10^{-5} M or more, where $D_{OH} = 5.23 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ was used.⁵⁶⁾ In view of the numerical values of the rate constants, i.e. $k_{1,w} = 2.4 \times 10^{-3} \text{ sec}^{-1}$ and $k_{1,OH} = 1.2 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$, the catalytic effect of water may be considered to be of secondary significance in the experimental conditions employed in this study. Namely, the use of Eq.(5-5) in place of Eq.(5-4) is justified for the present purpose.

The mean thickness of the reaction layer $\bar{\mu}$ which is now given by $\bar{\mu} = (D_{xy} / (k_{-1,OH}(OH^-)^0 + k_{-2,OH}(OH^-)^0))^{1/2}$, is calculated to be 9.3×10^{-4} cm when $(OH^-)^0 = 3.6 \times 10^{-5}$ M. $D_{xy} = 6.71 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$, $k_{-1,OH} = 8.4 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$, and $k_{-2,OH} = 11.5 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$. The numerical values of the rate coefficients used here were those obtained in the present study (see Table V-3). The magnitude of $\bar{\mu}$ is less than one twentieth of the thickness of the mean diffusion layer for hydroxyl ion, ($\delta \approx 2.3 \times 10^{-2}$ cm), which is an acceptable result. Similar arguments can also be applied to D-glucose and D-galactose.

Fig.V-3 shows a plot of $\log[(\bar{i}_1 - \bar{i})/\bar{i}^{3/2}]$ against E . The experimental points lie on a straight line, as expected from Eq.(5-10) with a reciprocal slope value of $2.303RT/\alpha_a n_a F \sim 100$ mV per log unit.

The general solution of the current potential curve of the kinetic wave in buffered solutions has been given by Koutecky.⁵⁷⁾ Rewriting his result for the present case, that is, for the current-potential curve of monosaccharide in buffered solutions, we obtain

$$E = E_o + \frac{RT}{\alpha_a n_a F} \ln[k_{el} (D_s (k_{-1} + k_{-2}))^{-1/2}] + \frac{RT}{\alpha_a n_a F} \ln\left[\frac{\bar{i}_1 - \bar{i}}{\bar{i}}\right] \quad (5-11)$$

Fig.V-4 shows a plot of $\log[(\bar{i}_1 - \bar{i})/\bar{i}]$ vs. E for the polarographic current of monosaccharides in buffered solution of pH 8.56. As expected from Eq.(5-11), a straight line was obtained. The value of the slope $2.303RT/\alpha_a n_a F$ was again ca. 100 mV; this result is a reasonable one provided that the discharge process of monosaccharides is identical both in buffered and in unbuffered solutions.

From Eq.(5-10), we obtain

$$E_{1/2} = E_o + \frac{RT}{\alpha_a n_a F} \ln\left[\frac{k_{el} (\bar{\kappa}_{OH})^{1/2}}{(D_s (k_{-1,OH} + k_{-2,OH}))^{1/2}}\right] - \frac{RT}{\alpha_a n_a F} \ln\left[\frac{\bar{i}_1}{2}\right]^{1/2} \quad (5-12)$$

for the half-wave potential of the polarographic wave in unbuffered solutions. Fig.V-5 shows the plot of $\log \bar{i}_1$ against $E_{1/2}$. As expected from Eq.(5-12), the data are distributed along a straight line with a reciprocal slope of $2.303RT/2\alpha_a n_a F \sim -50$ mV. The result that the values of $\alpha_a n_a$ are nearly identical for all monosaccharides investigated (Fig.V-3 and V-4) suggests the similarity of the discharge process for these monosaccharides.

In conclusion, the current-potential relationship of monosaccharide is fairly well reproduced by Eq.(5-10). It is to be noted here that in deriving Eq.(5-10), k_{el} is assumed to be independent of the concentrations of acid and/or base at the electrode surface. However, there is a reason to believe that the discharge process itself should depend on the concentrations of acid and/or base.

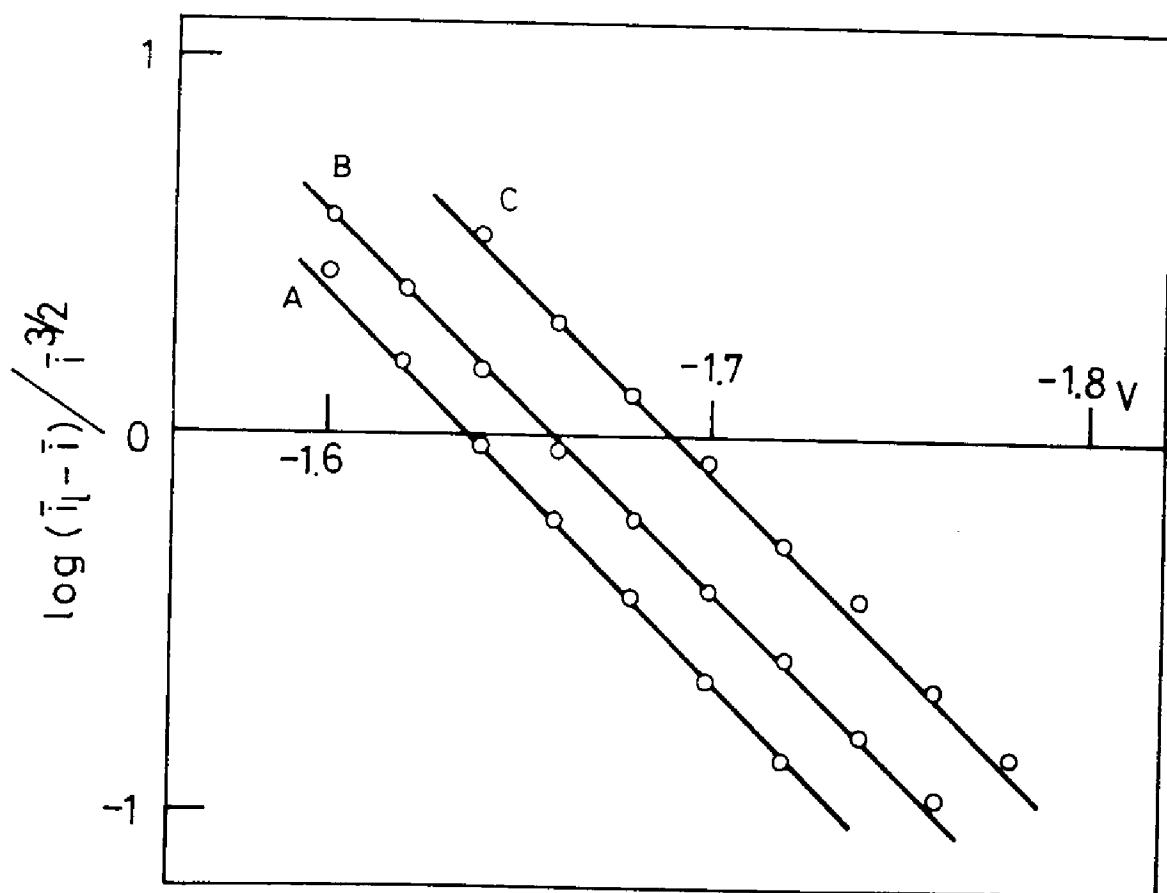


Fig.V-3 Plot of $\log[(\bar{i}_l - \bar{i})/\bar{i}^{3/2}]$ against E for the polarographic current of D-xylose (8 mm) A, D-glucose (40mm) B, D-galactose (12mm) C in 0.1 M potassium chloride solution at temp. 25°C. Reciprocal slope of the solid lines: 100 mV.

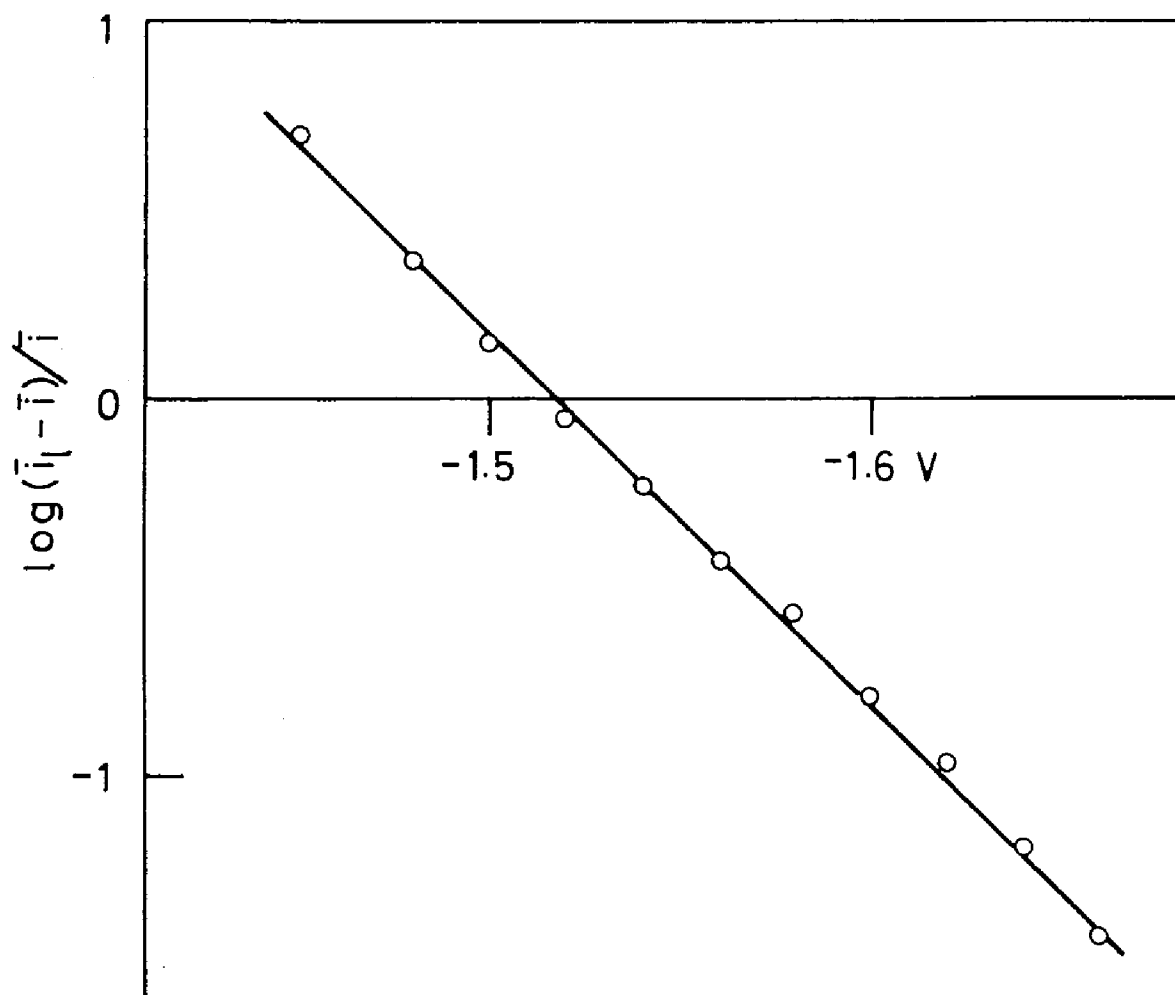


Fig.V-4 Plot of $\log[(\bar{i}_l - \bar{i})/\bar{i}]$ against E for the polarographic current of D-xylose (66mm) in 0.1M ammonia buffer of pH 8.56 and of ionic strength 0.5 at temp. 25°C. Reciprocal slope of the solid lines : 100 mV.

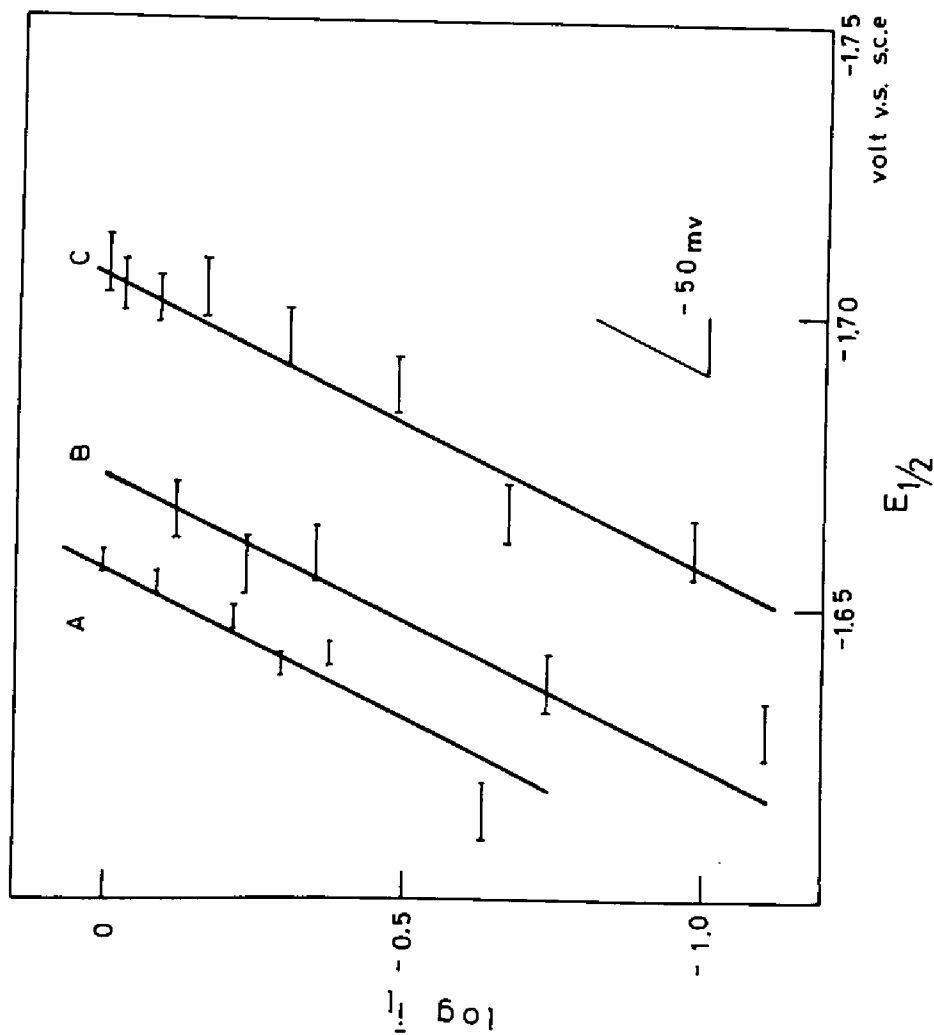


Fig.V-5 Plot of $\log i_L$ against $E_{1/2}$ for the polarographic current of D-xylose (A), D-glucose (B), D-galactose (C) in 0.1M potassium chloride solution at temp. 25°C. Reciprocal slope of the solid lines: -50 mV.

When TEAI was used as a supporting electrolyte, the half-wave potential of the monosaccharide appeared in more negative potential than in KCl solution. The reciprocal slope of the plot of $\log[(\bar{i}_1 - \bar{i})/\bar{i}^{3/2}]$ against E became 200 mV or even greater. A similar effect has also been observed^{23,24)} when tetramethyl-ammonium phosphate was used as the supporting electrolyte. This effect may be attributed to the adsorption of the quaternary ammonium cation on the electrode surface,⁵⁶⁾ which results in the retardation of the electro-reduction of monosaccharides.

DETERMINATION OF THE MUTAROTATION RATE CONSTANT

When a weighed amount of a monosaccharide in pure α -pyranose form was dissolved in 0.1M potassium chloride, the limiting current decreased with time and reached a constant value corresponding to the value for the equilibrated mixture of the monosaccharide. This behavior is very much similar to that in buffered solutions and the mutarotation rate constants may be obtained by analyzing the limiting current with the aid of Eq.(5-7) as a function of time.

When we start from α -pyranose, the change of concentrations of α - and β -pyranose with time is given as follows:⁸⁾

$$\begin{aligned} C_{\alpha} &= C_{\alpha,eq} (1 + K \exp(-k_{o,w}t)) \\ C_{\alpha} &= C_{\beta,eq} (1 - \exp(-k_{o,w}t)) , \end{aligned} \quad (5-13)$$

where $k_{o,w}$ is the overall mutarotation rate constant of water and is defined by four individual rate constants as follows:

$$k_{o,w} = \frac{(k_{1,w}k_{-2,w} + k_{-1,w}k_{2,w})}{(k_{-1,w} + k_{-2,w})} \quad (5-14)$$

Substitution of Eq.(5-13) into Eq.(5-7) yields:

$$\bar{i}_1(t)^{1/2} = (0.966 \bar{\kappa}_s (D_s/D_{OH})^{1/2} \tau)^{1/2} (C_{\alpha,eq} + C_{\beta,eq}) \times \\ \times \frac{[(k_{1,OH} + K k_{2,OH}) + K(k_{1,OH} - k_{2,OH}) e^{-k_{o,w} t}]}{(k_{-1,OH} + k_{-2,OH})^{1/2} (1+K)} \quad (5-15)$$

Subtraction of the expression corresponding with Eq.(5-15) at time $t + \Delta t$ and taking logarithms leads to the result

$$\ln(\bar{i}_1(t)^{1/2} - \bar{i}_1(t+\Delta t)^{1/2}) = \ln[(0.966 \bar{\kappa}_s (D_s/D_{OH})^{1/2} \tau)^{1/2} (C_{\alpha,eq} + C_{\beta,eq}) \times \\ \times \frac{K(k_{1,OH} - k_{2,OH}) (1 - e^{-k_{o,w} \Delta t})}{(k_{-1,OH} + k_{-2,OH})^{1/2} (1+K)}] - k_{o,w} t \quad (5-16)$$

Plotting $\log(\bar{i}_1(t)^{1/2} - \bar{i}_1(t+\Delta t)^{1/2})$ with constant Δt vs. t , according to the method of Guggenheim,⁵⁸⁾ should give a straight line with slope $-k_{o,w}$, and intercept $= \ln[(0.966 \bar{\kappa}_s (D_s/D_{OH})^{1/2} \tau)^{1/2} K \times (k_{1,OH} - k_{2,OH}) (C_{\alpha,eq} + C_{\beta,eq}) (1 - \exp(-k_{o,w} \Delta t))] / [(1+K) (k_{-1,OH} + k_{-2,OH})^{1/2}]$ (5-17)

At $t \rightarrow \infty$, Eq.(5-15) is reduced to

$$\bar{i}_1(t \rightarrow \infty)^{1/2} = (0.966 \bar{\kappa}_s (D_s/D_{OH})^{1/2} \tau)^{1/2} \times \\ \times \frac{(C_{\alpha,eq} + C_{\beta,eq}) (k_{1,OH} + k_{2,OH})}{(k_{-1,OH} + k_{-2,OH})^{1/2} (1+K)} \quad (5-18)$$

Namely, the overall catalytic rate constant, $k_{o,w}$ is determined from the slope of the Guggenheim plot. Furthermore, if the overall catalytic rate coefficient of hydroxyl ion, $k_{o,OH}$ and the equilibrium constant, K , are known, Eqs.(5-17) and (5-18) in combination with the following two equations

$$k_{o,OH} = (k_{1,OH}k_{-2,OH} + k_{-1,OH}k_{2,OH}) / (k_{-1,OH} + k_{-2,OH}) \quad (5-19)$$

$$K = C_{\beta,eq} / C_{\alpha,eq} = k_{1,OH}k_{-2,OH} / k_{-1,OH}k_{2,OH} \quad (5-20)$$

can be solved with respect to four individual rate coefficients, $k_{1,OH}$, $k_{2,OH}$, $k_{-1,OH}$, and $k_{-2,OH}$.

An example of the Guggenheim plot according to eq.(5-16) is shown in Fig.V-6. The values of $k_{o,w}$ determined by the present method are given in the second column of Table V-2. The results are in good accordance with those obtained by the polarimetric method (the third column in Table V-2).

TABLE V-2. THE OVERALL MUTAROTATION RATE CONSTANT $k_{o,w}$, IN 0.1 M KCl SOLUTION AT 25°C

	$k_{o,w} \text{ (sec}^{-1}\text{)}$	
	Polarography	Polarimetry
D-glucose	1.31×10^{-3}	1.3×10^{-3}
D-galactose	0.53×10^{-3}	0.4×10^{-3}
D-xylose	0.54×10^{-3}	0.5×10^{-3}

TABLE V-3. THE FORWARD AND BACKWARD RATE COEFFICIENTS, $k_{i,OH}$ (i = 1, 2, -1, AND -2) AT 25°C

	$k_{1,OH}$	$k_{2,OH}$	$k_{-1,OH}$	$k_{-2,OH}$
		$(M^{-1} \text{sec}^{-1})$		
D-glucose	11.6×10	6.5×10	9.1×10^4	8.9×10^4
D-xylose	8.2×10^2	6.0×10^2	8.4×10^4	11.5×10^4

Table V-3 shows the individual rate coefficients of hydroxyl ion for D-xylose and D-glucose, $k_{o,OH} = 7 \times 10^2 M^{-1} \text{sec}^{-1}$ (D-xylose),

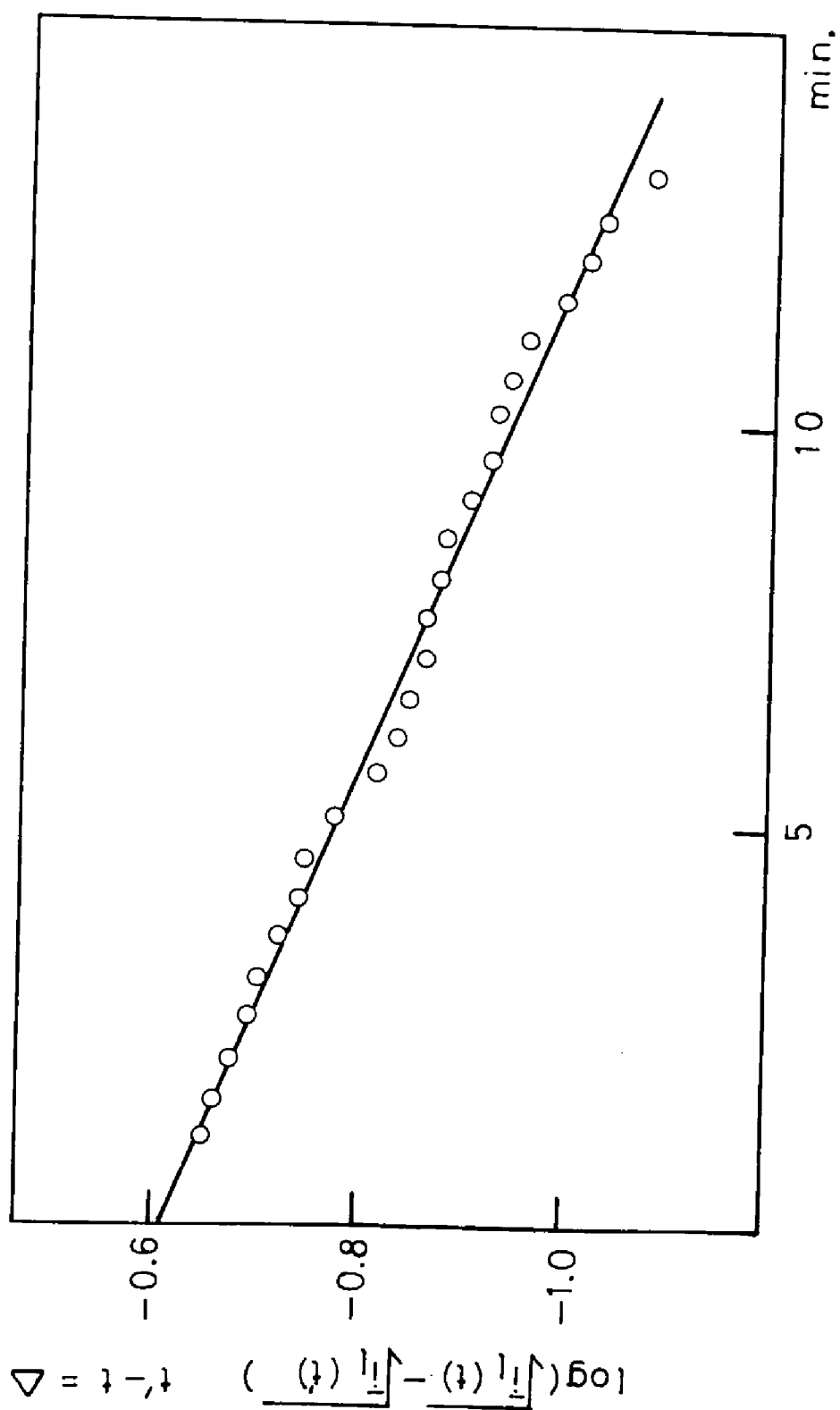


Fig.V-6 Analysis of current vs. time curve when α -D-xylose is dissolved at time $t = 0$ in 0.1 M potassium chloride solution.

$9 \times 10^{-6} \text{ M}^{-1} \text{ sec}^{-1}$ (D-glucose)¹⁶⁾, $D_{xy} = 6.7 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$, $D_{glu} = 6.65 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$,⁴⁵⁾ $K_{xy} = 1.87$ and $K_{glu} = 1.74$ ¹⁴⁾ being used. The forward rate coefficients, $k_{1,OH}$ and $k_{2,OH}$, for D-xylose are in fair agreement with those obtained from the analysis of the polarographic currents in buffered media (see Table III-5). The ratio of the concentration of the intermediate γ -form, $C_{\gamma,eq}$ to that of α - and β -pyranose form, $C_{\alpha,eq} + C_{\beta,eq}$, was $3 \times 10^{-1} \%$ for D-xylose and $4 \times 10^{-2} \%$ for D-glucose. These results are also in the same order as those obtained from the experiments in basic buffer solutions (see Table II-3).

SUMMARY

The polarographic behavior of monosaccharides in unbuffered neutral solution was investigated. The limiting current of monosaccharides in unbuffered solution was ascertained to be an autocatalytic current which could be caused by the production of hydroxyl ion at the electrode surface. The equation for the shape of the current-potential curve as well as the equation for the limiting current was derived by use of the reaction layer concept. Experimental results for several monosaccharides (D-glucose, D-galactose, and D-xylose) were fairly well expressed by these equations. The wave can also be applied to the determination of the mutarotation rate constants of monosaccharides. The values of the mutarotation rate constants determined by this method were in fair agreement with those obtained by the polarimetric method.

CONCLUSION

In this study the polarographic behavior of monosaccharides in aqueous solutions was investigated with intent to shed light on the kinetics of the mutarotation reaction.

First, the polarographic behavior of α - β -equilibrated mixture of D-glucose, D-galactose, and D-xylose were studied in an ammonia buffer solution. Hydrolysis, carbonyl amino reaction, or any other complicated reactions of these monosaccharides did not occur under the conditions used in the present experiment (pH<9.5, Temp. 25°C). The dependence of the limiting current on the concentration of monosaccharides, temperature, the height of mercury reservoir, the pH, and the concentration of buffer components was investigated. The polarographic limiting current had a kinetic character, but it was inclined to increase slightly with increasing height of the mercury reservoir. The limiting current depended on the concentration of the buffer component as well as the pH of the experimental solution. These results suggest that the equilibrium concentration of the reducible intermediate given by the reaction scheme (pyranose \rightleftharpoons γ -form (reducible intermediate)) is very small in comparison with the concentration of pyranose form; and the velocity of the reaction is catalyzed by the buffer components as well as hydroxyl ions. The controlled-potential electrolysis of the monosaccharides and paper-chromatography of electrolyzed products revealed that D-glucose, D-galactose, and D-xylose are reduced to sorbitol, dulcitol, and xylitol, respectively at the mercury cathode in weakly basic solution.

Wiesner's equations, relating the kinetic current with four rate constants of the equation; α -pyranose $\xrightleftharpoons[k_{-1}]{k_1}$ γ -form $\xrightleftharpoons[k_2]{k_{-2}}$ β -pyranose were modified so that the equations can be applied for the case of the smaller values of the rate constants. By the use of these modified equations, the individual and overall rate constants

were determined for several monosaccharides in neutral phosphate and basic ammonia buffer solutions. The ratio of the concentration of the intermediate γ -form to the concentration of the pyranose form was calculated. The value of this ratio depended on both kinds and concentrations of the buffer salts. The ratio in ammonia buffer was about 10 times larger than that in phosphate buffer; and it decreased with increasing concentration of buffer salts.

The rate constants of the mutarotation of α -D-xylose were determined in various buffer solutions. The overall and the forward rate constants were analyzed as a linear function of the buffer concentration. It was revealed that the catalytic effects of the buffer salts are mainly due to their basic components for all the buffer salts investigated. The Brönsted plots of k_1 and k_2 for all the basic components gave straight lines with the same slope of 0.42. The rate constants for several amines deviated from the slope; this deviation was ascribed to the steric hindrance caused by the large molecular sizes.

The activation free energies, enthalpies, and entropies of the mutarotation of α -D-xylose in a neutral phosphate buffer solution were determined. Experimental results suggest a reaction mechanism containing several acyclic intermediates for the mutarotation of D-xylose.

The mechanism of the acid-base catalyzed mutarotation of monosaccharide was discussed on the basis of the above experimental results. A probable mechanism is proposed, in which four intermediates are assumed.

The polarographic behavior of monosaccharides in unbuffered aqueous solution was investigated. The limiting current was ascertained to be an autocatalytic current which could be caused by the production of hydroxyl ion at the electrode surface. Equations for the shape of the current-potential curve and for the limiting current were derived by use of the reaction layer concept.

Experimental results for several monosaccharides were fairly well expressed by these equations. The wave was successfully applied to determine the mutarotation rate constants of monosaccharides. The values of the mutarotation rate constants determined by this method were in fair agreement with those obtained by the polarimetric method.

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